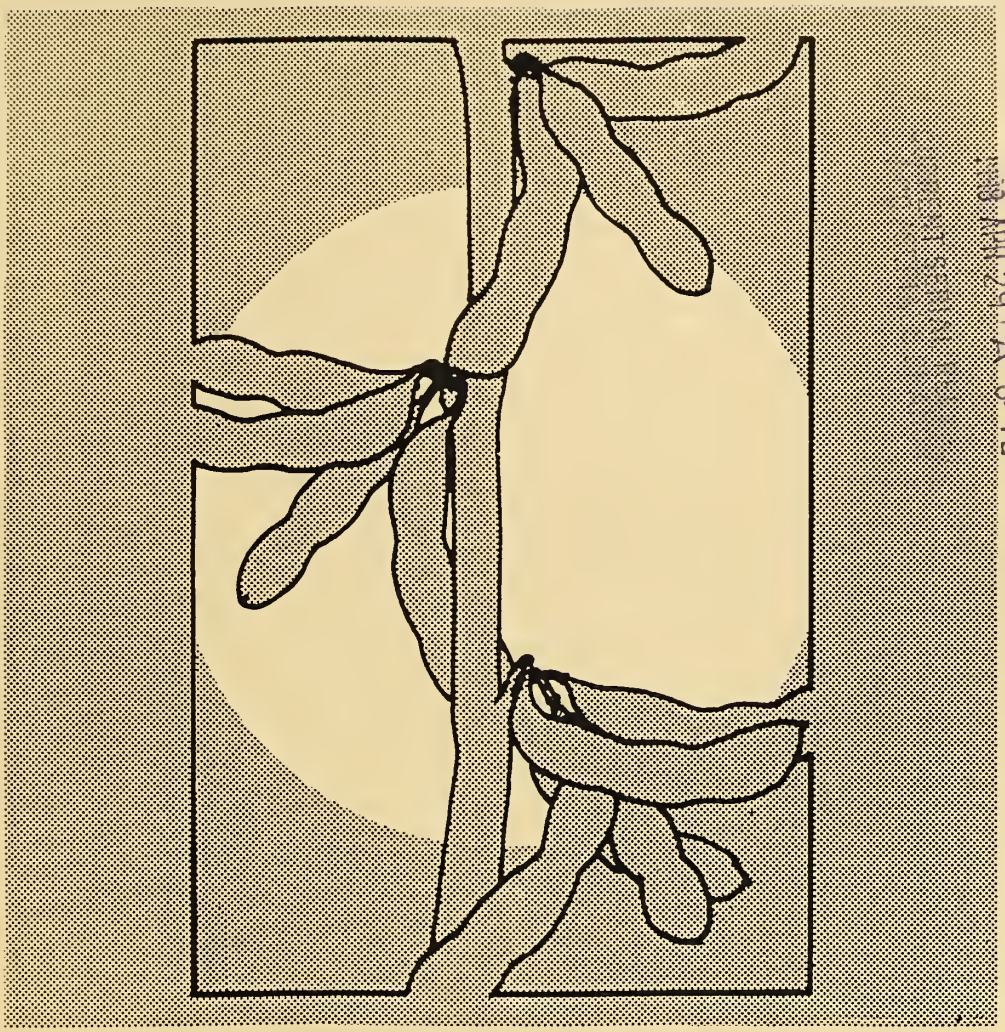
Soybean Genetics Newsletter



Volume 23 May 1996

The data presented here are not to be used in publications without the consent of the respective authors.

USDA-Agricultural Research Service
Department of Agronomy
and Department of Zoology / Genetics
Iowa State University
Ames, Iowa 50011

Table of Contents

Brazil

Canada

The People's Republic of China

Czech Republic

India

Japan

Pakistan

Poland

Thailand

The United States of America

Iowa

Illinois

Louisiana

Michigan

Minnesota

Missouri

North Carolina

Ohio

South Carolina

Tennessee

Virginia

Washington

Foreword

Volume 23, 1996 of the Soybean Genetic Newsletter has been possible by the concerted effort of Tracy Dang, USDA Biological Laboratory Technician, and Ivy Bode, an undergraduate Entomology major at Iowa State University.

Several articles deserve special mention;

We sincerely appreciate our international contributors and wish for even more contributions for Volume 24. The continued support from our U. S. contributors is encouraging.

Reid G. Palmer, editor
USDA-ARS-FCR

Data presented in the Soybean Genetics Newsletter are not to be used in publications without the consent of the respective authors.

Mention of a trademark or proprietary product by the USDA or Iowa State University does not imply its approval to the exclusion of other products that may also be suitable.

NEW BOOK

Soybean: Genetics, Molecular Biology and Biotechnology

Edited by D PS Verma, Plant Biotechnology Center, The Ohio State University, and R.C. Shoemaker, Department of Agronomy, lowa State University, USA

Biotechnology in Agriculture Series, No.14.

Soybean is one of the world's leading sources of seed oil and seed protein and is grown extensively, particularly in the Americas and Asia. Indeed, soybean is the lowest-cost producer of vegetable oil, since the oil is a coproduct of its protein-rich meal. Major advances have recently been made in our understanding of soybean genetics and of the application of new technologies to soybean improvement. Thus it is now possible, using molecular methods, to alter the protein and oil composition of soybean, as well as produce other foreign proteins in the plant. Further progress should permit the improvement of nitrogen fixation and other desirable traits. This book focuses on recent progress in our understandin of the genetics and molecular biology of soybean and provides a broad review of the subject, from genome diversity to transformation and integration of desired genes using current technologies. It is aimed at workers in legume agronomy, plant genetic, breeding and biotechnology.

Contents:

Readership: Workers in legu~ne agronomy, plant genetics, breeding and biotechnology.

October 1995 288 pages HB
ISBN 0 85198 984 5
Price: £49.95 (US$90.00 Americas

CAB INTERNATIONAL
Headquartes, Wallingford, Oxon OX10 8DE, UK. Tel: (01491) 832111 Telex: 847964 (COMAGG G) Fax: (01491) 826090
North America, 2001 Evans Rd., Cary, North Carolina 27513, USA. Tel: 919/677-0977 800/445-9714 Fax: 919/677-1303
Africa, PO Box 76520, Nairobi, Kenya. Tel: Nairobi 747 340/337 Telex: 22040 ILRAD (KE) (for IIBC Muguga) Fax: Nairobi 747 340
Asia, PO Box 11872, 50760 Kuala Lumpur, Malaysia. Tel (03) 255 2922 Telex: 28031 (MA CABI) Fax: (03) 255 1888
Caribbean, Gordon Street, Curepe, Trinidad & Tobago. Tel 809 662 4173 Telex: 24438 (CARIRI) Fax: 809 663 2859

ORDER FORM - BOOKS

❒ Yes, I would like to order _____copies of
Soybean: Genetics, Molecular Biology and Biotechnology

£49.95 (US$90.00 Americas only)

❒ I enclose payment of £/$_____

❒ Please send a proforma invoice

Postage • packing - no charge in UK. For prepaid orders elsewhere, please add £2.00 for 1st book and 60p for each additional book ordered (up to 5). Maximum postage charge is £4.20. For orders not pre-paid, postage and packing will be charged according to the weight of the book.

❒ Please charge against my credit card, my number is _____
Expiry date _____

❒ Access/Mastercard ❒ Visa ❒ Amex *(Please tick relevant box)*

Address of cardholder if different from below:

_____

_____

Important - European Union Countries only
Are you registered for VAT (BTW/MWST/TVA/MOMS/FPA/TAV/MWS/ALV/MOM) Yes/No
Number_____

Surname_____Initials _____Dr/Mr/Mrs/Ms
Organization_____
Address_____

_____

_____

Post/Zip Code _____ Country _____
Date _____ Signed _____
Telephone No: _____ Fax No. _____

Please return your order to any of the addresses overleaf
or give your credit card details by fax or phone
Fax: 01491 833508 Tel: 01491 832111

Aug 1995/1854

USDA SOYBEAN GERMPLASM COLLECTION REPORT

In 1995, a total of 29,992 seed lots were distributed from the USDA Soybean Germplasm Collection in response to 333 requests. This large increase in seed distribution was due to extensive plantings for disease screening in 1995.

In 1995, we began cooperative projects to screen the Collection for sources of resistance to several diseases. These seed shipments accounted for 18,404 of the seed lots that we sent out during the year. We sent 11,392 packets of seed representing nearly 4,680 accessions from maturity groups 000 through III to seven cooperators in Minnesota, Michigan, Ohio, and Wisconsin to be screened for resistance to Sclerotinia whitemold. We sent seed of approximately 4,440 accessions from maturity groups IV through VIII to Louisiana for screening for resistance to aerial web blight and 1,605 accessions from maturity groups 00 through IV to Ohio for screening for Phytophthora rot resistance. Finally, one cooperator at the University of Illinois screened 965 accessions for resistance to Septoria brown spot.

For seed replacement of *G. max* accessions in 1995, four-row plots were planted at three locations: 778 plots at Urbana, 92 plots at Stoneville, and 319 plots last winter at Isabela, Puerto Rico. Only one accession of *G. soja* was grown in Urbana for seed replacement. General field evaluation of germplasm was conducted at Urbana, Stoneville, Isabela, and Rosemont, Minnesota this year. At Urbana, we completed the second year of evaluation for 1031 accessions in maturity groups I through IV. The planting at Rosemont was the second year of evaluation for groups 000 through I accessions. The Urbana and Rosemont tests contain new accessions received since 1987, including the lines obtained from central China in 1992. At Stoneville, we also completed the second year of evaluation for the 854 accessions in maturity groups VII and VIII.

We had 3,226 plots planted in 1995 for the purpose of purelining new accessions. Of these, 543 were grown at Urbana, 2,455 were grown at Stoneville, and 154 at Isabela. After the process is completed this spring, we expect to add over 900 accessions to the Collection. Most of the pureline rows planted in Stoneville were from the second (1994) germplasm exchange with China. These came from Anhui, Fujian, Guangdong, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, and Zhejiang provinces. The purelines grown in Urbana were also mostly from China but came from many different donors. In addition, there were also accessions from Japan and Russia.

We have received 54 new accessions from Japan and South Korea that will be planted in 1996. We are anticipating another 500 germplasm accessions from south and central China in the spring of 1996.

Final data for the evaluation of 814 accessions of maturity group VI which was begun at Stoneville in 1992 have been collected. The data are currently being entered into the computer and will be tabulated and published in a USDA technical bulletin. After the conclusion of the winter growing season in Puerto Rico, we will begin preparing the groups IX and X evaluation data for publication in a USDA technical bulletin. Pedigree information on Northern Uniform Soybean Tests from 1939 to 1990, co-authored by R. L. Bernard, S. K. St. Martin, J. R. Wilcox, and P. I. Morgan, was published in November as USDA-ARS Technical Bulletin 1846. The text of the Evaluation of Maturity Groups 000 - IV (FC 01.547 - PI 266.807) was approved for publication in October 1995 and will be published as a USDA-ARS Technical Bulletin.

Li Zhenglu, a visiting scholar from the Crop Research Institute of the Shandong Academy of Agricultural Science in Jinan, Shandong,China, is currently working with the germplasm project.

Jerry Hill began work as the Assistant Curator of the Collection in March of 1995. He has made operational our new database system which uses the "Access" database software. This year, we will begin using the system for tracking new introductions through the two year process of purelining and final entry in the collection. This local database has greatly increased the ease and flexibility of order entry and printing, data entry and modification, inventory tracking, planting preparation, and fieldbook production. Another important accomplishment Jerry has made is to connect us with the Internet. This has made it possible to increase the speed of our connection with the GRIN computer while eliminating long distance phone charges. In addition to uploading order information via this route, we have also begun to upload and download accession and inventory data in the same way.

Our Collection database currently contains data on the following entries:

Subcollection	**Entries**
Introduced *G. max*	13,375
G. soja	1,097
Germplasm releases	116
Modern cultivars	368
Old cultivars	210
Private cultivars	35
Williams isolines	100
Clark isolines	295
Harosoy isolines	126
Other isolines	43
Genetic types	151
Total	15,916

The following information was taken from Dr. Ted Hymowitz' annual report for the specific cooperative agreement "Management of the USDA perennial *Glycine* Germplasm Collection". The current inventory of the perennial *Glycine* consists of 887 accessions of 16 species. Of these, 779 accessions in 14 species are available for distribution and 722 accessions in 12 species are stored at the National Seed Storage Laboratory in Ft. Collins. Fifty seeds of 78 accessions were sent to the National Seed Storage Laboratory in 1995. During the 1995 report year, 672 seed packets were sent in response to 32 requests from 11 states and 3 foreign countries. Most packets shipped had 5 or 10 seeds per packet.

J. L. Hill and R. L. Nelson
USDA- Agricultural Research Service
USDA Soybean Germplasm Collection
11011 W. Peabody Drive
Urbana, Illinois 61801

1996 Soybean Crop Germplasm Committee Meeting

The Soybean Crop Germplasm Committee (formerly Soybean Germplasm Crop Advisory Committee) held its annual meeting February 19, 1996 in conjunction with the Soybean Breeders' Workshop at St. Louis, MO. In attendance were committee members Thomas Devine, John Thorne, Lawrence Young, Thomas Kilen, John Hicks, Bill Kenworthy, Doyle Ashley, Terry Niblack, Randall Nelson, H. Roger Boerma, Gary Ablett, Emerson Shipe, Jerry Hill, John All, Edgar Hartwig, Dan Phillips, and Robert Freestone, and guests, Tommy Carter and Mark Bohning. New members Robert Freestone, Terry Niblack, and Emerson Shipe were introduced. Chair, Tom Devine, called the meeting to order at 8:00 a.m.

A draft report entitled **Soybean Crop Germplasm Committee Report, 1996** was sent to each member prior to the meeting for discussion by each subcommittee. This report is being prepared for the ARS National Program Staff. The committee broke into subcommittees until 9:30 a.m. The subcommittee reports are as follows:

Acquisition (Kenworthy, Chair; Phillips, Hill, Young, Hartwig)

The acquisition sub-committee was in general agreement with the draft report outlining germplasm needs for G. max, G. soja, and the perennial Glycine species. Recent acquisitions from China have greatly increased the representation from Central and South China in the G. max collection. The U.S. should continue to monitor the Glycine accessions in other national collections from India, Japan, S. Korea, and Russia for potential new accessions which are not in the current collection. Accessions from North Korea are under represented in the collection.

Field collection for G. max is desirable in certain areas of South Asia. The Committee agrees that Vietnam should be a high priority for field collection because of the great diversity of primitive varieties still in production in that country.

It was also suggested that enhanced germplasm from any active breeding or improvement programs in the Asian region would also merit collection or exchange.

The G. soja collection is small compared to the G. max collection. The Committee agrees that G. soja accessions from China, S. Korea, Russia, and Japan would be valuable additions to the collection. Several specific islands around S. Korea and Japan which would be good sites for collection. The Committee also felt that efforts to identify the outer fringe of the distribution area of G. soja would be fertile collection areas. The priority should be to focus on exchange of G. soja accessions already existing in several national collections and in individual scientist's collection.

The perennial Glycine species are represented in the collection, and a 1996 collection trip to Australia should provide additional accessions. The Agricultural Research Service is providing $15,000 to support this expedition. The Committee felt that these species should receive lesser emphasis than the G. max and G. soja collections. The perennials are difficult and expensive to

maintain because they require greenhouse facilities for grow-outs. The cooperative relationship with Australia has been productive and successful in expanding the number of perennial Glycine accessions in the collection. This cooperation should be encouraged and continued.

The Committee agrees with the relative priority ranking of germplasm acquisition in the proposal. Germplasm exchange has been and remains the key method of acquisition and should be pursued. The committee was supportive of the need for additional staff. If more accessions are obtained and result in over loading the system, the entire collection may be placed at risk. Therefore, staffing needs must be addressed to facilitate the expansion of the collection that has occurred and is anticipated.

Evaluation, Crop Vulnerability (Kilen, Chair; All, Freestone, Niblack)

To conduct valid and repeatable systematic evaluation of germplasm for pest resistance, there is a need for standardized assays and access to pure cultures or colonies of pest organisms.

In addition to current ongoing evaluation projects, some specific needs are:

I. Evaluations for which techniques are established, but which may be expensive to conduct.

1. Soybean Rust
2. Northern Root Knot Nematode
3. Silverleaf Whitefly

II. Evaluations for which techniques are not well developed, or the pest organisms are composed of several variants.

1. Frogeye Leafspot
2. Charcoal Rot
3. Stinkbug pod feeding
4. Corn earworm and other lepidopterous pod feeders
5. Bean leaf beetle pod feeding

Additionally, it would be desirable to characterize pest resistant germplasm lines for molecular markers that are closely linked to loci involving traits with complex inheritance. Marker assisted selection could be used in breeding programs to transfer useful traits from primitive accessions. Requisite assay of pests would be needed to verify pest resistance loci as a phenotypic screen. These markers could also be used to indirectly select for resistance to pests such as soybean rust that require expensive containment-facility evaluation. The improved efficiency of evaluation and selection might promote greater utilization of the germplasm collection, and greater genetic diversity.

Enhancement (Boerma, Chair; Ashley, Devine, Hicks, Thorne)

The subcommittee discussed the draft report and the following paragraph was suggested and approved:

"Recent research clearly demonstrates the limited genetic base of the U.S. soybean crop. Extensive germplasm exchange among programs has been the key factor for continuous incremental yield gains. As the impact of commercial varieties increases in the market place, and variety patenting becomes commonplace, germplasm exchange and yield advances are threatened as never before. Public field-oriented programs must expand their role in the creation of germplasm with desirable combinations of genetically diverse alleles for agronomically important traits. The ability of new molecular technologies to improve previously intractable traits i.e.(high yield & drought tolerance) will require continued field evaluations prior to use by commercial breeding organizations".

Operations (Nelson, Chair; Ablett, Bohning, Shipe)

The subcommittee discussed the problems that increased seed requests, large additions of new accessions, and management of large databases create for the Collection. Additional staff are needed to handle the current responsibilities and to continue to expand the size of the Collection.

Other Business

Nelson and Kilen were assigned to revise the Soybean Crop Germplasm Committee Report for National ARS program staff.

Devine reported that two requests have been submitted to the committee for inclusion of Recombinant Inbred Lines in the soybean Germplasm Collection: 284 lines of the cross, Minsoy x Noir 1 and 147 lines for the cross PI290136 x BARC-2 (Rj4).

Work is continuing on the development of a core collection for soybean germplasm.

Nelson distributed the annual USDA Soybean Germplasm Collection Report. Jerry Hill began work as the Assistant Curator of the Collection in March, 1995. It is expected that about 500 new accessions from south and central China will be received and planted in 1996, as well as 54 new accessions from Japan and South Korea, which were received after planting in 1995.

Mark Bohning distributed a progress report on GRIN. User access to GRIN is now available through the Internet, resulting in much higher use rates than before.

A proposal to add a member to the committee representing the soybean industry was discussed. A motion was made to appoint a subcommittee (Kenworthy, Shipe and Hicks) to develop a plan to add such a representative, probably from the United Soybean Board.

Devine expressed appreciation to retiring members, Young, Boerma, and Thorne.

Devine was elected Chair for 1996. Shipe was elected Vice Chair.

Meeting adjourned at 11:45 p.m.

T. E. Devine, Chair
John Thorne, Vice-chair

Soybean Crop Germplasm Committee Members

February 19, 1996

Member	Area of Representation	Term Expires
Ablett, Gary Ridgetown College of Agricultural Technology Ridgetown, Ontario NOP2CO Ph: 519-674-1635 FAX: 519-674-1600	Canadian Representative	1997
All, John University of Georgia Department of Entomology Athens, Georgia 30602-7503 Ph: 706-542-7589 FAX: 706-542-2279 E-Mail: jall@bugs.ent.uga.edu	Entomology	1997
Ashley, Doyle A. University of Georgia Department of Crops Soil Science 3111 Plant Sciences Bldg. Athens, GA 30602 Ph: 706-542-0922 FAX: 706-542-0914	Physiology	1998
Devine, Thomas E., Chair USDA, ARS, PSI, PMBL Bldg. 006, Rm 118, BARC-W 103000 Baltimore Ave. Beltsville, Maryland 20705-2350 Ph: 301-504-6375 FAX: 301-504-5320	Cytogenetics and Molecular Genetics	1997
Freestone, Robert Pioneer Hi-Bred 3261 W. Airline Highway Waterloo, IA 50703 Ph: 319-234-0335 FAX: 319-233-6850	Private Breeding, North	1999

Member	Area of Representation	Term Expires
Hartwig, Dr. Edgar Soybean Production Research USDA, ARS P. O. Box 196 Stoneville, Mississippi 38776 Ph: 601-686-3126 FAX: 601-686-3140	USDA Germplasm Collection	Ex Off.
Hicks, John D. Pioneer Hi-Bred International P. O. Box 4428 Seven Oaks Road Greenvill, Mississippi 38704 Ph: 601-335-9152 FAX: 601-335-9164	Private Breeding, South	1998
Hill, Jerry USDA-ARS University of Illinois 1101 West Peabody Urbana, IL 61801 Ph: 217-244-4346 FAX: 217-333-4639 E-Mail: j-hill26@ux1.cso.uiuc.edu	USDA Germplasm Collection	Ex. Off.
Kenworthy, William J. University of Maryland Department of Agronomy College Park, Maryland 20742 Ph: 301-405-1324 FAX: 301-314-9041 E-Mail: wk7@umail.umd.edu	Public Breeding, North	1997
Kilen, Thomas C. Soybean Production Research USDA, ARS P. O. Box 196 Stoneville, Mississippi 38776 Ph: 601-686-3125 FAX: 601-686-3140 E-Mail: a03sprstone@attmail.com	USDA Germplasm Collection	Ex Off.

Member	Area of Representation	Term Expires
Nelson, Randall University of Illinois Department of Agronomy, NSRL, USDA 1101 West Peabody Urbana, IL 61801 Ph: 217-244-4346 FAX: 217-333-4639	USDA Germplasm Collection	Ex. Off
Niblack, Terry Department of Plant Pathology 108 Waters Hall University of Missouri Columbia, MO 65211 Ph: 573-882-7333 FAX: 573-882-0588 E-Mail: terry-niblack@mvccmail.missouri.edu	Nematology	1999
Phillips, Daniel Plant Pathology University of Georgia 1109 Experiment Station Griffin, GA 30223 Ph: 404-412-4009 FAX: 404-228-7203	Plant Pathology	1998
Shipe, Emerson Agronomy Department. Clemson University Box 340359 Clemson, SC 29634-0359 Ph: 864-656-3524 FAX: 864-656-3443	Public Breeding, South	1999

Soybean Genetics Committee Report

The Soybean Genetics Committee met February 19, 1996 at the Sheraton Inn, St. Louis, Missouri in conjunction with the National Soybean Breeders' Workshop. Committee members attending the meeting were: G. R. Buss, B. Diers, R. L. Nelson, J. H. Orf, T. W. Pfeiffer, J. E. Specht, and S. St. Martin. Glenn Buss and Steve St. Martin had been elected by mail ballot to serve a three-year term on the committee. Todd Pfeiffer was reelected Chair for the year ending in February, 1997. Current committee members and February expiration dates for their terms on the committee are:

Dr. G. R. Buss (1999)
Dept. of Crop/Soil/Env. Sciences
VPI and State University
Blacksburg, VA 24061
Tel: (540) 231-9788
Fax: (540) 231-3431
E-mail: gbuss@vt.edu

Dr. B. Diers (1997)
Dept. of Crop/Soil Sciences
Michigan State University
E. Lansing, MI 48824
Tel: (517) 353-4587
Fax: (517) 353-5174
E-mail: 22903mgr@msu.edu

Dr. B. R. Hedges (1998)
Pioneer Hi-Bred Production Ltd.
P. O. Box 730, No. 2 Highway West
Chatham, Ontario, N7M5L1
Canada
Tel: (519) 436-6729
Fax: (519) 436-6741
E-mail: hedgesbr@phibred.edu

Dr. R. L Nelson (ex officio)
USDA-ARS, Turner Hall- Agronomy
1102 South Goodwin
University of Illinois
Urbana, IL 61801
Tel: (217) 244-4346
Fax: (217) 333-4639
E-mail: rnels@ux1.cso.uiuc.edu

Dr. J. H. Orf (1997)
Dept. Agronomy/Plant Genetics
University of Minnesota
St. Paul, MN 55108
Tel: (612) 625-8275
Fax: (612) 625-1268
E-mail: orfxx001@maroon.tc.umn.edu

Dr. R. G. Palmer (ex officio)
USDA-ARS
Dept. Agronomy/Genetics
Iowa State University
Ames, IA 50011
Tel: (515) 294-7378
Fax: (515) 294-2299
E-mail: rpalmer@iastate.edu

Dr. T. W. Pfeiffer(1998) (Chair)
Department of Agronomy
University of Kentucky
Lexington, KY 40546
Tel: (606) 257-4678
Fax: (606) 323-1952
E-mail: agr044@ukcc.uky.edu

Dr. S. K. St. Martin (1999)
Department of Agronomy
2021 Coffey Rd.
Ohio State University
Columbus, OH 74078
Tel: (614) 292-8499
Fax: (614) 292-7162
E-mail: sstmarti@magnus.acs.ohio-state.edu

Procedure

As in the past, manuscripts concerning qualitative genetics interpretation, gene symbols, and linkages should be sent to the Chairperson of the Soybean Genetics Committee for review. To facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol. "Manuscripts will not be reviewed for style except as this influences the clarity of interpretation. Manuscripts will not be "peer reviewed" unless requested by the author. Authors may submit unpolished (but comprehensible) manuscripts for review, unless peer review is requested. This should reduce delays involved in publishing a paper.

2. Reviewers of manuscripts will be given a deadline of three weeks to return the reviewed manuscript to the Chairman (who will then return it to the author as soon as possible). If the reviewers have not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairman of the Soybean Genetics Committee.

Assignment/Approval of Gene Symbols

If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R. L. Nelson is Curator for all maturity groups. A form for this purpose is on page 21 of this volume.

Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretation does not depend upon symbols, in most cases.) The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is not necessary.

New Business

The committee voted to recommend that the statement on the cover of the Soybean Genetics Newsletter "The data presented here are not to be used in publications without the consent of the respective authors" be changed to "Articles in the SGN may be cited unless the authors has designated that prior approval must be obtained." The change is proposed for the 1997 SGN, and the recommendation will be sent to Reid Palmer, editor, SGN.

Discussion items:

Naming of genes underlying QTL and naming genes for cDNA probes of defined genes in other organisms: The naming of genes underlying QTL must follow the "Guidelines on the Evidence Necessary for the Assignment of Gene Symbols". The assignment of a gene symbol will depend on the strength of the expression of the alternate phenotypes as opposed to the strength of the linkage. Similarly, to name a gene for a defined cDNA probe from another organism which maps to the soybean molecular map, alternate phenotypes for the expression of that gene must be demonstrated and inheritance shown following the "Guidelines on the Evidence Necessary for the Assignment of Gene Symbols".

It was suggested that if data which result in the assignment of a gene symbol are later proven to be incorrect, the gene symbol is dropped and not reassigned.

Gene symbols assigned March 1995 - February 1996.

Date	**Authors**	**Trait**	**Gene Symbol/Linkage**
8 Jan 1996	Kosslak Chamberlain Palmer	Disease lesion mimic - root necrosis	*rn* (Ames 1) *rn* (Ames 2) *rn* (Ames 3)
21 Aug 1995	Thompson Bernard Nelson	Tall determinate growth	*dt1-t*
2 Aug 1995	Kollipara Singh Hymowitz	Protease inhibitors in *G. tomentella*	*Pi1 pi1* *Pi2 pi2* *Pi3 pi3*
30 June 1995	Nelson	Branching pattern	*Br1 br1* *Br2 br2*
10 May 1995	Chen Palmer	Tan saddle seed coat Malate dehydrogenase Linkage Group (not assigned)	*k2* (Columbia 1) *k2* (Columbia 2) *Mdh1-n* (Columbia) *Mdh1-n* (Ames 5) *Mdh1-n* (Ames 6) *k2-Mdh1* 1±1.36 cM
27 Dec 1995	Ilarslan Skorupska Horner Palmer	Male and female sterility	*st6* and *st7*
15 Feb 1995	Cober Voldeng	Linkage Group 5	*E3 - Dt1* 27.5 ± 3.2 cM
22 Feb 1996	Sneller Isleib	Linkage Group 15	*Pgm1 - ms2* 18.7 ± 2.4 cM

Guidelines on the Evidence Necessary for the Assignment of Gene Symbols

Researchers are strongly encouraged to send all gene symbols and genetic interpretations to the Soybean Genetics Committee for review prior to publication to avoid duplication and/or confusion. Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing.

The following is a set of guidelines prepared by the Soybean Genetics Committee and intended to help researchers undertaking genetic analysis of soybean traits. Of necessity, these procedures will often need to be modified by the researcher to fit the specific situation, but an application of these guidelines should aid in making the correct genetic interpretation.

1. A genetic hypothesis is made on the basis of classification of segregating progeny, usually the F_2 generation and here called the hypothesis generation.

2. A second generation with a pedigree trace to the first generation, is classified to confirm the proposed genetic hypothesis. This second generation may be progeny of the hypothesis generation (usually F_3) or progeny of a testcross (F_1 x recessive homozygote).

3. Traits that are strongly influenced by nongenetic factors require verification of the classification scheme by evaluation of the progeny from homozygous plants of the hypothesis generation. Testcross data are not suitable for this purpose.

4. For genes controlling a phenotypic expression similar to that of previously published genes, data must be obtained to test for uniqueness and allelism. This will usually require crossing a homozygous line carrying the newly identified gene with the original sources of the previously published genes. If appropriate allelism tests are not included in a manuscript, the committee will request such information from the researcher. Molecular linkages can also be used to demonstrate that the allelism test conducted is the only one needed.

5. Identification of cytoplasmic factors requires reciprocal crosses between parents differing in the trait of interest. Since these factors are transmitted through the cytoplasm, the trait is expected to be associated only with the maternal parent in the F_1 and succeeding generations. Maternal effects need to be distinguished from cytoplasmic effects by using reciprocal F_1 and F_2 data.

6. Conclusive evidence for cytoplasmic factors should rule out self pollinations and nongenetic factors associated with the maternal parent. Selecting parents for reciprocal crosses that differ in nuclear genetic traits (e.g., flower or pubescence color) in addition to possible cytoplasmic traits will provide evidence of cross- rather than self-pollinations by observed segregation for the nuclear genetic trait in succeeding generations.

7. Inheritance patterns in a hypothesis generation (F_2) and a confirming generation (F_3) are absolute requirements for differentiating between cytoplasmic factors and nuclear genetic traits.

8. Follow the guidelines (Rules for Genetic Symbols) published in the Soybean Genetics Newsletter to assign the symbol.

9. Submit the manuscript to the chair, Soybean Genetics Committee, for review of the genetic interpretation and approval of the gene symbol (see Soybean Genetics Newsletter for name and address). Please indicate in unequivocal terms your willingness to provide seed for allelism tests requested by researchers discovering genes with a similar phenotype. This does not restrict your asking for a signature on a Material Transfer Agreement.

10. If the line in which the new gene occurs in not already in the USDA Germplasm Collection, you are strongly encouraged to send a seed sample of the line to the curator of the Genetic Type Collection for assignment of a T-number and maintenance of the seed (see the current Soybean Genetics Newsletter for name and address).

References

Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombinational values in heredity. Hilgardia 24:235-278.

Hanson, W. D. 1959. Minimum family size for the planning of genetic experiments. Agron. J. 51:711-715.

Immer, F. R. 1930. Formulae and tables for calculating linkage intensities. Genetics 15:81-98.

Immer, F. R. and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-410.

Mather, K. 1951. The measurement of linkage in heredity. Methuen and Co., Ltd. London. John Wiley and Sons, Inc. New York.

Sedcole, J. R. 1977. Number of plants necessary to recover a trait. Crop Sci. 17:667-668.

Rules for Genetic Symbols

I. Gene Symbols

a. Gene symbols will not be assigned to traits for which no inheritance data are presented.

b. A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below. Gene symbols may, however, be written on one line.

c. Genes that are allelic shall be symbolized with the same base letter(s) so that each genetic locus will be designated by a characteristic symbol base.

d. Gene pairs that govern the same phenotype (including duplicate, complementary or polymorphic genes) constitute multiple loci that should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: Y_1, Y_2, etc.) The numerals may be written on the same line as the base. (Example: *Y1, Y2*, etc.) This shall be the only use of numerals. Letter designations should not be used. The numeral 1 is automatically a part of the first reported gene symbol for each base but may be omitted only until the second symbol is assigned.

e. The first pair of alleles reported for a genetic locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: *Ab, ab*; *Ab* is allelic and dominant to *ab*.)

f. If two alleles are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion and the alleles may be differentiated by adding one or two uncapitalized letters as superscripts to the base. When more than two alleles exist for a locus, the additional alleles, or those symbolized subsequently to the pair first published, shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: *R*, r^m, *r*.) This shall be the only use of superscripts. The letters may be written on the same line as the base if preceded by a hyphen. (Example: *Rps1-b, Rps1-k*, and *Ap-a, Ap-b, Ap-c*.) The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The letters may be an abbreviation of a descriptive term.

If independent mutations with the same or similar phenotype are identified at the same locus, until it is possible genetically to ascertain if they represent identical or separate alleles, the gene symbol should be followed by an identifying designation in parentheses. The identifying designation, which should **NOT** be in italics or underlined, can be the place where the mutation was found, the cultivar in which it was found, or any other relevant characteristic of the mutation. (Example: *ms1* [Tonica], or *ms1* [Ames 2].) This will ensure that possible subtle differences between the mutations, such as differences in DNA sequence, or unique pleiotropic side effects, are not overlooked by workers using those genes.

g. Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.

h. The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clearcut. The decision for intermediate cases is at the discretion of the author, but should be in accordance with previous practices for the particular type of trait.

i. An underscore may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A_ represents both AA and Aa.)

j. A question mark may be used in place of a symbol when the locus or allele is unknown or doubtful. The name of the line in which the gene was identified should be included in the symbol, in parentheses. A hyphen preceding the question mark indicates an unknown allele at a known locus, the absence of a hyphen indicates an unknown locus. (Example: *Rps?* [Harosoy] an allele in Harosoy at an unknown locus or *Ap-?* [T160] an unknown allele in T160 at the *Ap* locus.)

k. Plus (+) symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

II. Isoenzyme Symbols and Protein Gene Symbols

The following set of guidelines is to be used when assigning gene symbols to isoenzyme variants. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybeans.

a. A gene symbol (generally three letters) that indicates, as clearly as possible, the name of the enzyme should be used. (Example: Adh [alcohol dehydrogenase], Idh [isocitrate dehydrogenase].) The appropriate Enzyme Commission name and number should be used in the original article, when appropriate, to designate the specific enzyme activity being investigated.

b. The electrophoretic conditions used to characterize a locus or allele should be specified clearly and in sufficient detail to be repeated by others interested in using the locus in genetic studies. The electrophoretic mobility, or other properties of an allele, should be clearly described by the authors.

c. Publications should include a photograph and/or an interpretive zymogram that allows readers to visualize the variability described by the authors, as well as to ensure that subsequent work corresponds to the original study.

III. Probe detected loci

The following guidelines are to be used for assigning locus names to probe-detected (RFLP) loci. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybeans.

a. Locus designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names. (Example: Iowa St. Univ., IaSU). The prefix shall not be necessary in publications except as needed to distinguish numbers that would, without the prefix, be identical.

b. The prefix is followed by a string of letters and/or integers that identify the probe used to detect the locus by the originating laboratory. This probe-identifying string should be limited to no more than six characters. This string should be separated from the prefix by a hyphen. (Example: IaSU-B317).

c. The probe-identifying sting is followed by the restriction endonuclease used in the restriction digest of the soybean genomic DNA that was probed. The following abbreviations for restriction enzymes are recommended: EcoRI = I, EcoRV = V, HinddIII = H,DraI = D, RsaI = R, Bc1I = B, TaqI = T. (Example: IaSU-B317I, IaSU-B317T).

d. Duplicate loci detected by the same probe should be identified with the same letter and integer base differentiated by integers (1, 2, 3, 4, etc.) consecutively assigned in the order of publication. These numerals are to be separated from the base string by a hyphen. Example: IaSU-B317I-1, IaSU-B317T-2, etc.)

e. Upon publication of new RFLP loci, researchers are strongly encouraged to
   1. make the probe identifying the locus/loci publicly available
   2. make available the identity of the restriction endonuclease used to generate the mapped polymorphism
   3. make available the identity of the genetic stock used to map the locus/loci
   4. make available the molecular weights of the polymorphic fragments used to map the locus/loci

IV. Random Amplified Polymorphic DNA (RAPD) loci

The following guidelines are to be used in assigning names to loci that are mapped using RAPD technology. The system adopted here is that which is generally employed in other species in which RAPD loci have been mapped.

a. Locus designations should begin with a letter identifying the origin of the primer. (Example: Operon Technologies, O)

b. The origin of the primer is followed by the primer name. (Example: Primer number 14 from Operon Technologies kit A, OA14.)

c. The primer name is followed in subscript by the fragment size in base pairs of the amplified fragment that is being mapped. (Example: An 800 bp fragment amplified with Operon Technologies primer 14 from kit A, OA14800).

V. Simple sequence repeat (SSR) or microsatellite loci

a. Locus designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names. (Example: Iowa St. Univ., IaSU). The prefix shall not be necessary in publications except as needed to distinguish numbers that would, without the prefix, be identical.

b. The prefix should be followed by a string of letters that identify the core nucleotide repeat of the SSR followed by an identifying number. This string should be separated from the prefix by a hyphen and should not exceed eight characters. (Example: IaSU-at275, BARC-gata3412).

c. Upon publication of new SSR loci, researchers are strongly encouraged to
   1. make available the oligonucleotide primers sequences required for amplification of the SSR
   2. make available the identity of the genetic stock used to map the locus/loci.

VI. Linkage and Chromosome Symbols

a. Linkage groups and the corresponding chromosomes shall be designated with Arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.

b. Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc. The symbol Def shall denote deficiencies, Inv, inversions; and Tri, primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second Def 1b, etc. The first published inversion in chromosome 1 shall be designated with the Arabic numeral that corresponds to its respective linkage group number.

c. Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2 the second case, etc. The first published deficiency shall be symbolized as Def A, the Def B, etc. The first published inversion shall be symbolized as Inv A, and the second as

Inv B, etc. The first published trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

VII. Cytoplasmic Factor Symbols

a. Cytoplasmic factors shall be designated with one or more letters prefixed by *cyt-*. (Example: *cyt-G* indicates the cytoplasmic factor for maternal green cotyledons, *cyt-Y* indicates that for maternal yellow cotyledons.)

b. Designations for specific cytoplasmic factors following *cyt-*, shall follow the same format as for gene symbols. Base letters chosen to indicate apparent relationships among traits will have common initial letters for all loci in a related group of traits. Initial letters will be consistent with initial letters designating nuclear gene traits. (Example: *cyt-G* green seed embryo, *cyt-Y2* yellow leaves, becoming yellowish green.)

VIII. Priority and Validity of Symbols

a. A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.

b. In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

IX. Rule changes

a. These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

APPLICATION FOR ENTRY INTO THE SOYBEAN GENETIC TYPE COLLECTION

Date: _____ T number (assigned by curator) _____

Submitted by: _____

Address: _____

_____

_____

_____

_____

_____

_____

Return to:
R.L. Nelson, curator
USDA Soybean Germplasm Collection
Department of Agronomy
University of Illinois
1102 South Goodwin Avenue
Urbana, Illinois, 61801, U.S.A.

Strain Designation: _____

Genotype: _____

Phenotype: _____

_____

Parental Origin: _____

_____

_____

_____

When and where found and by whom: _____

_____

_____

_____

Description

Maturity Group _____ Stem termination _____

Pubescence color _____ Pubescence type and density _____

Seed coat luster and color _____ Hilum color _____

Flower color _____ other _____

Pod color _____ _____

Special instructions for growing or maintenance, if any: _____

_____

Literature References: _____

_____

_____

(List the reference(s) that first and best describe the discovery and inheritance of the trait. Please send relevant reprints to the curator.

Date seedlot received at Urbana: _____ Date T number assigned: _____

USDA/ARS/FIELD CROPS RESEARCH
Department of Agronomy
Department of Zoology/Genetics
Iowa State University
Ames, Iowa 50011
USA

Qualitative Genetic Traits

A comprehensive tabulation of qualitative genetic traits with gene symbols, phenotypic description, source of mutant, and references was presented in the *Soybean Monograph* (Palmer and Kilen, 1987). A listing of gene symbols, with phenotype and references that have been approved by the Soybean Genetics Committee since 1986, is presented in Tables 1 to 11. These data illustrate that since 1986, much emphasis in soybean genetics has been on disease resistance, seed components, and isoenzymes and proteins. These tables are adapted from a book chapter that we prepared for the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia.

Reid G. Palmer - USDA

Randy C. Shoemaker - USDA

REFERENCES

Amberger, L. A., R. C. Shoemaker, and R. G. Palmer. 1992. Inheritance of two independent isozyme variants in soybean plants derived from tissue culture. Theor. Appl. Genet. 84:600-607.

Anderson, T. R., and R. I. Buzzell. 1992. Inheritance and linkage of the *Rps7* gene for resistance to *Phytophthora* rot of soybean. Plant Dis. 76:958-959.

Boerma, H. R., and D. V. Phillips. 1983. Genetic implications of the susceptibility of Kent soybean to *Cercospora sojina*. Phytopathology 74:1666-1668.

Bowers, Jr., G. R., K. Ngeleka, and O. D. Smith. 1993. Inheritance of stem canker resistance in soybean cultivars Crockett and Dowling. Crop Sci. 33:67-70.

Bult, C. J., and Y. T. Kiang. 1989. Inheritance and genetic linkage tests of an esterase locus in the cultivated soybean, *Glycine max*. J. Hered. 80:82-85.

Buzzell, R. I., and T. R. Anderson. 1992. Inheritance and race reaction of a new soybean *Rps1* allele. Plant Dis. 76:600-601.

Buzzell, R. I., and B. R. Buttery. 1992. Inheritance of an anomalous flavonoid glycoside gene in soybean. Genome 35:636-638.

Buzzell, R. I., and J.C. Tu. 1984. Inheritance of soybean resistance to soybean mosaic virus. J. Hered. 75:82.

Buzzell, R. I., and J. C. Tu. 1989. Inheritance of soybean stem-tip necrosis reaction to soybean mosaic virus. J. Hered. 75:400-401.

Chandlee, J. M., and L. O. Vodkin. 1989. Unstable expression of a soybean gene during seed coat development. Theor. Appl. Genet. 77:587-594.

Chen, P., G. R. Buss, C. W. Roane, and S.A. Tolin. 1991. Allelism among genes for resistance to soybean mosaic virus in strain-differential soybean cultivars. Crop Sci. 31:305-309.

Chen, X. F., and R. G. Palmer. (nd). Inheritance and linkage with the *k2* and *Mdh1-n* loci in soybean. J. Hered. (In press)

Chiang, Y. C., M. B. Gorman, and Y. T. Kiang. 1987. Inheritance and linkage analysis of phosphoglucose isomerase isozymes in soybeans. Biochem. Genet. 25:893-900.

Chiang, Y. C., and Y. T. Kiang. 1988. Genetic analysis of mannose-6-phosphate isomerase in soybeans. Genome 30:808-811.

Cianzio, S. R., and R. G. Palmer. 1992. Genetics of five cytoplasmically inherited yellow foliar mutants in soybean. J. Hered. 83:70-73.

Devine, T. E., and L. D. Kuykendall. 1994. Genetic allelism and linkage tests of a soybean gene, *Rfg1*, controlling nodulation with *Rhizobium fredii* strain USDA 205. Plant Soil 158:47-51.

Doong, J. Y. H., and Y. T. Kiang. 1987a. Inheritance of soybean endopeptidase. Biochem. Genet. 25:847-853.

Doong, J. Y. H., and Y. T. Kiang. 1987b. Inheritance of aconitase isozymes in soybean. Genome 29:713-717.

Doong, J. Y. H., and Y. T. Kiang. 1988. Inheritance study on a soybean fluorescent esterase. J. Hered. 79:399-400.

Erickson, E. A., J. R. Wilcox, and J. F. Cavins. 1988. Inheritance of altered palmitic acid percentage in two soybean mutants. J. Hered. 79:465-468.

Fehr, W. R., G. A. Welke, E. G. Hammond, D. N. Duvick, and S. R. Cianzio. 1991. Inheritance of reduced palmitic acid content in seed oil of soybean. Crop Sci. 31:88-89.

Fehr, W. R., G. A. Welke, E. G. Hammond, D. N. Duvick, and S. R. Cianzio. 1992. Inheritance of reduced linolenic acid content in soybean genotypes A16 and A19. Crop Sci. 32:903-906.

Foley, T. C., R. L. Nelson, and C. D. Nickell. 1996. Inheritance of dwarfness from soybean strain T281. Crop Sci. (In press)

Gorman, M. B., and Y. T. Kiang. 1978. Models for the inheritance of several variant soybean electrophoretic zymograms. J. Hered. 69:255-258.

Graef, G. L., W. R. Fehr, and E. G. Hammond. 1985. Inheritance of three stearic acid mutants of soybean. Crop Sci. 25:1076-1079.

Graybosch, R. A., and R. G. Palmer. 1987. Analysis of a male-sterile character in soybeans. J. Hered. 78:66-70.

Griffin, J. D., and R. G. Palmer. 1986. An additional beta-amylase mobility variant conditioned by the *Sp1* locus. Soybean Genet. Newsl. 13:150-151.

Griffin, J. D., and R. G. Palmer. 1987. Inheritance and linkage studies of five isozyme loci in soybean. Crop Sci. 27:885-893.

Griffin, J. D., and R. G. Palmer. 1989. Genetic studies with two superoxide dismutase loci in soybean. Crop Sci. 29:968-971.

Hanson, P. M., C. D. Nickell, L. E. Gray, and S. A. Sebastian. 1988. Identification of two dominant genes conditioning brown stem rot resistance in soybean. Crop Sci. 28:41-43.

Harper, J. E., and C. D. Nickell. 1995. Genetic analysis of nonnodulating soybean mutants in a hypernodulated background. Soybean Genet. Newsl. 22:185-190.

Hedges, B. R., and R. G. Palmer. 1992. Inheritance of malate dehydrogenase nulls in soybean. Biochem. Genet. 30:491-502.

Hildebrand, D. F., and T. Hymowitz. 1980. Inheritance of β-amylase nulls in soybean seeds. Crop Sci. 20:727-730

Honeycutt, R. J., J. W. Burton, R. C. Shoemaker, and R. G. Palmer. 1989. Expression and inheritance of a shriveled-seed mutant in soybean. Crop Sci. 29:704-707.

Honeycutt, R. J., K. E. Newhouse, and R. G. Palmer. 1990. Inheritance and linkage studies of a variegated leaf mutant in soybean. J. Hered. 81:123-126.

Ilarslan, H., H. T. Skorupska, H. T. Horner, and R. G. Palmer. (nd). Cytology and genetics of a tisssue-culture derived soybean genic male-sterile. (Submitted).

Keen, N. T., and R. I. Buzzell. 1991. New disease resistance genes in soybean against *Pseudomonas springae* pv. *glycinea*: Evidence that one of them interacts with a bacterial elicitor. Theor. Appl. Genet. 81:133-138.

Kiang, Y. T. 1981. Inheritance and variation of amylase in cultivated and wild soybeans and their wild relatives. J. Hered. 72:382-386.

Kiang, Y. T., and C. J. Bult. 1991. Genetic and linkage analysis of aconitate hydratase variants in soybean. Crop Sci. 31:322-325.

Kiang, Y. T., Y. C. Chiang, and C. J. Bult. 1987. Genetic study of glutamate oxaloacetic transaminase in soybean. Genome 19:370-373.

Kiihl, R. A. S., and E. E. Hartwig. 1979. Inheritance of reaction to soybean mosaic virus in soybeans. Crop Sci. 19:372-375.

Kilen, T. C., and E. E. Hartwig. 1987. Identification of single genes controlling resistance to stem canker in soybean. Crop Sci. 27:863-864.

Kollipara, K. P., R. J. Singh, and T. Hymowitz. 1996. Inheritance of protease inhibitors in *Glycine tomentella* Hayata (2n=38), a perennial relative of soybean. J. Hered. (In press).

Kosslak, R. M., J. D. Dieter, R. L. Ruff, M. C. Chamberlin, B. A. Bowen, and R. G. Palmer. 1996. Tolerance to root-borne infection by *Phytophthora sojae* in three allelic necrotic-root mutants in soybean. J. Hered. (In press).

Lim, S. M. 1989. Inheritance of resistance to *Peronospora manshurica* races 2 and 33 in soybean. Phytopathology 79:877-879.

Lohnes, D. G., and R. L. Bernard. 1992. Inheritance of resistance to powdery mildew in soybeans. Plant Dis. 76:964-965.

Luzzi, B. M., H. R. Boerma, and R. S. Hussey. 1994. A gene for resistance to the southern root-knot nematode in soybean. J. Hered. 85:484-486.

Ma, G., P. Chen, G. R. Buss, and S. A. Tolin. 1995. Genetic characteristics of two genes for resistance to soybean mosaic virus in PI 486355 soybean. Theor. Appl. Genet. 91:907-914.

McBlain, B. A., and R. L. Bernard. 1987. A new gene affecting the time of maturity in soybeans. J. Hered. 78:160-162.

Nelson, R. L. 1996. The inheritance of a branching type in soybean. Crop Sci. 36:(In press).

Palmer, R. G. 1984. Pleiotropy or close linkage of two mutants in soybean. J. Hered. 75:457-462.

Palmer, R. G., and R. W. Groose. 1993. A new allele at the *w4* locus derived from the *w4-m* mutable allele in soybean. J. Hered. 84:297-300.

Palmer, R. G., and T. C. Kilen. 1987. Qualitative genetics and cytogenetics. In J. R. Wilcox (ed.) Soybeans: Improvement, production and uses. Agronomy 16:135-209.

Palmer, R. G., and H. Skorupska. 1990. Registration of a male-sterile genetic stock (T295H) of soybean. Crop Sci. 30:241.

Palmer, R. G., B. R. Hedges, R. S. Benavente, and R. W. Groose. 1989. *w-4*-mutable line in soybean. Devel. Genet. 10:542-551.

Palmer, R. G., R. L. Nelson, R. L. Bernard, and D. M. Stelly. 1990. Genetics and linkage of three chlorophyll-deficient mutants in soybean: *y19*, *y22*, and *y23*. J. Hered. 81:404-406.

Pfeiffer, T. W., D. F. Hildebrand, and J. H. Orf. 1993. Inheritance of a lipoxygenase - 1 allozyme in soybean. Crop Sci. 33:691-693.

Phillips, D. V., and H. R. Boerma. 1982. Two genes for resistance to race 5 of *Cercospora sojina* in soybeans. Phytopathology 72:764-766.

Pracht, J. E., C. D. Nickell, and J. E. Harper. 1993. Genes controlling nodulation in soybean: *Rj5* and *Rj6*. Crop Sci. 33:711-713.

Ray, J. D., K. Hinson, J. E. B. Mankono, and M. F. Malo. 1995. Genetic control of a long-juvenile trait in soybean. Crop Sci. 35:1001-1006.

Reese, Jr., P. F., and H. R. Boerma. 1989. Additional genes for green seed coat in soybean. J. Hered. 80:86-88.

Rennie, B. D., W. D. Beversdorf, and R. I. Buzzell. 1987a. Genetic and linkage analysis of an aconitate hydratase variant in soybean. J. Hered. 78:323-326.

Rennie, B. D., W. D. Beversdorf, and R. I. Buzzell. 1987b. Inheritance and linkage analysis of two endopeptidase variants in soybeans. J. Hered. 78:327-328.

Rennie, B. D., and J. W. Tanner. 1989a. Comparison of the *fan* alleles in C1640 and the lines A5, PI 123440, and PI 361088B. Soybean Genet. Newsl. 16:23-25.

Rennie, B. D., and J. W. Tanner. 1989b. Genetic analysis of low linolenic acid levels in the line PI 123440. Soybean Genet. Newsl. 16:25-26.

Rennie, B. D., J. Zilka, M. M. Cramer, and W. D. Beversdorf. 1988. Genetic and linkage analysis of low linolenic acid levels in the soybean line PI 361088B. Crop Sci. 28:655-657.

Roane, C. W., S. A. Tolin, and G. R. Buss. 1983. Inheritance of reaction to two viruses in the soybean cross 'York' x 'Lee 68'. J. Hered. 74:289-291.

Rode, M. W., and R. L. Bernard. 1975. Inheritance of a tan saddle mutant. Soybean Genet. Newsl. 2:39-42.

Sawada, S., and R. G. Palmer. 1987. Genetic analyses of nonfluorescent root mutants induced by mutagenesis in soybean. Crop Sci. 27:62-65.

Schnebly, S. R., W. R. Fehr, G. A. Welke, E. G. Hammond, and D. N. Duvick. 1994. Inheritance of reduced and elevated palmitate in mutant lines of soybean. Crop Sci. 34:829-833.

Sebastian, S. A., and R. S. Chaleff. 1987. Soybean mutants with increased tolerance for sulfonylurea herbicides. Crop Sci. 27:948-952.

Sebastian, S. A., G. M Fader, J. F. Ulrich, D. R. Forney, and R. S. Chaleff. 1989. Semidominant soybean mutation for resistance to sulfonylurea herbicides. Crop Sci. 29:1403-1408.

Seo, Y. W., J. E. Specht, G. L. Graef, and R. A. Graybosch. 1993. Inheritance of red-buff seed coat in soybean. Crop Sci. 33:754-758.

Skorupska, H., and R. G. Palmer. 1989. Genetics and cytology of the *ms6* male-sterile soybean. J. Hered. 80-304-310.

Skorupska, H. T., and R. G. Palmer. 1990. Additional sterile mutations in soybean, *Glycine max* (L. Merr.) J. Hered. 81:296-300.

Stephens, P. A., and C. D. Nickell. 1992. Inheritance of pink flower in soybean. Crop Sci. 32:1131-1132.

Stephens, P. A., C. D. Nickell, and F. L. Kolb. 1993. Genetic analysis of resistance to *Fusarium solani* in soybean. Crop Sci. 33:929-930.

Thompson, W. A., R. L. Bernard, and R. L. Nelson. (nd). A third allele at the soybean *dt1* locus. (Submitted)

Tu, J. C., and R. I. Buzzell. 1987. Stem-tip necrosis: A hypersensitive, temperature dependent, dominant gene reaction of soybean to infection by soybean mosaic virus. Can. J. Plant Sci. 67:661-665.

Werner, B. K., J. R. Wilcox, and T. L. Housley. 1987. Inheritance of an ethyl methanesulfonate-induced dwarf in soybean and analysis of leaf cell size. Crop Sci. 27:665-668.

Wilcox, J. R., and J. F. Cavins. 1987. Gene symbol assigned for linolenic acid mutant in the soybean. J. Hered. 78:410.

Wilcox, J. R., and T. S. Abney. 1991. Inheritance of a narrow, rugose-leaf mutant in *Glycine max*. J. Hered. 82:421-423.

Willmot, D. B., and C. D. Nickell. 1989. Genetic analysis of brown stem rot resistance in soybean. Crop Sci. 29:672-674.

Yee, C. C., J. Li, and Z. G. Yu. 1986. Genetic studies with Shennong 2015, a lethal yellow mutant (*y21*) in soybean. Hereditas (Beijing) 8:13-16.

Yu, H., and Y. T. Kiang. 1993a. Genetic characterization of a leaf margin necrosis mutant in wild annual soybean (*Glycine soja*). Genetica 90:31-33.

Yu, H., and Y. T. Kiang. 1993b. Inheritance and genetic linkage studies of isozymes in soybean. J. Hered. 84:489-492.

Table 1. Genes affecting pigmentation in soybean[a].

Gene	Phenotype	Reference
K2	Yellow seed coat	Rode and Bernard (1975); Palmer (1984)
k2 (T239)[b]	Tan saddle on seed coat	
k2 (Urbana) *y20* (Urbana) *Mdh1-n* (Urbana) (T253)	Tan saddle on seed coat, yellow-green plant, malate dehydrogenase null	Hedges and Palmer (1992)
k2 (Columbia 1) *Mdh1-n* (Columbia 1) (T261)	Tan saddle on seed coat, malate dehydrogenase null	Chen and Palmer (nd)
k2 (Columbia 2) (L67-3483)[c]	Tan saddle on seed coat	
*R**	Black seed coat with brown hilum	Chandlee and Vodkin (1989)
t-r (T236)	Red-buff seed coat	Seo et al. (1993)
w4-dp (T321)	Dilute-purple flower	Palmer and Groose (1993)
w4-m (T322)	Mutable flower color	Palmer et al. (1989)
wp	Pink flower	Stephens and Nickell (1992)

[a] References since 1986, except where previous references are necessary to describe the phenotype or the genetics of the trait.
[b] T-numbers are in the Genetic Type Collection.
[c] L67-3483 is in the Isoline Collection, cultivar Clark.

Table 2. Genes affecting herbicide reaction in soybean.

Gene	Phenotype	Reference
Als1	Semidominant for resistance to sulfonylurea herbicides	Sebastian et al. (1989)
als1	Sensitive	
Hs1	Sensitive to sulfonylurea herbicides	Sebastian and Chaleff (1987)
hs1	Enhanced tolerance	
Hs2	Sensitive to sulfonylurea herbicides	Sebastian and Chaleff (1987)
hs2	Enhanced tolerance	
Hs3	Sensitive to sulfonylurea herbicides	Sebastian and Chaleff (1987)
hs3	Enhanced tolerance	

Table 3. Genes affecting *Bradyrhizobium* or *Rhizobium* response in soybean.

Gene	Phenotype	Reference
Rfg1	Ineffective by strain 205	Devine and Kuykendall (1994)
rfg1	Effective	
Rj5	Nodulating	Pracht et al. (1993)
rj5	Nonnodulating	
Rj6	Nodulating	Pracht et al. (1993)
rj6	Nonnodulating	
Rj7	Nodulating	Harper and Nickell (1995)
rj7	Hypernodulating	
Rn (Ames 1)	Normal	Kosslak et al. (1996)
rn (Ames 1)	Necrotic root	
Rn (Ames 2)	Normal	
rn (Ames 2)	Necrotic root	
Rn (Ames 3)	Normal	
rn (Ames 3)	Necrotic root	

Table 4. Genes causing sterility in soybean[a].

Gene	Phenotype	Reference
Ms1	Male fertile	Skorupska and Palmer (1990)
ms1 (Ames 2) (T287H)[b]	Male sterile, female fertile	
ms1 (Danbury) (T290H)	Male sterile, female fertile	
Ms3	Male fertile	Graybosch and Palmer (1987); Skorupska and Palmer (1990)
ms3 (Flanagan) (T284H)	Male sterile, female fertile	
ms3 (Plainview) (T291H)	Male sterile, female fertile	
Ms4	Male fertile	Skorupska and Palmer (1990)
ms4 (Fisher) (T292H)	Male sterile, female fertile	
Ms6	Male fertile	Palmer and Skorupska (1990); Skorupska and Palmer (1989)
ms6 (T295H)	Male sterile, female fertile	
St6St6St7St7	Male fertile, female fertile	Ilarslan et al. (nd)
St6st6st7st7 (T331H)	Male sterile, female sterile	

[a] For T-strains (Genetic Type Collection) with an H suffix (eg., T287H), the allele is carried as the heterozygote because the homozygote is sterile, lethal, or very weak.

[b] T-numbers are in the Genetic Type Collection.

Table 5. Genes controlling inheritance of isoenzyme and protein variants in soybean[a].

Gene	Phenotype	Reference
Aco1-a	Aconitase mobility variant	Griffin and Palmer (1987); Kiang and Bult (1991)
Aco1-b	Aconitase mobility variant	
aco1-n	Aconitase null	
Aco2-a	Aconitase mobility variant	Doong and Kiang (1987b); Rennie et al. (1987a)
Aco2-b	Aconitase mobility variant	
Aco2-bn (T318)[b]	Aconitase null	Amberger et al. (1992)
Aco2-c	Aconitase mobility variant	Kiang and Bult (1991)
Aco3-a	Aconitase mobility variant	Griffin and Palmer (1987)
Aco3-b	Aconitase mobility variant	
Aco4-a	Aconitase mobility variant	Griffin and Palmer (1987)
Aco4-b	Aconitase mobility variant	
Aco4-c	Aconitase mobility variant	
Aco4-d	Aconitase mobility variant	
Aco5-a	Aconitase mobility variant	Kiang and Bult (1991)
Aco5-b	Aconitase mobility variant	
aco5-n	Aconitase null	
Adh3	Alcohol dehydrogenase present	Yu and Kiang (1993b)
adh3	Alcohol dehydrogenase absent	
Enp-a	Endopeptidase mobility variant	Doong and Kiang (1987a); Griffin and Palmer (1987); Rennie et al. (1987b)

Table 5. Continued.

Gene	Phenotype	Reference
Enp-b	Endopeptidase mobility variant	
Enp-c	Endopeptidase mobility variant	
Est1-a	Esterase mobility variant	Bult and Kiang (1989)
Est1-b	Esterase mobility variant	
Fle	Fluorescent esterase present	Doong and Kiang (1988)
fle	Fluorescent esterase absent	
Got-a	Glutamate oxaloacetate transaminase mobility variant	Kiang et al. (1987)
Got-b	Glutamate oxaloacetate transaminase mobility variant	
Got-c (T289)	Glutamate oxaloacetate transaminase mobility variant	
Lx1-b	Lipoxygenase-1 mobility variant	Pfeiffer et al. (1993)
Mdh1-n (Urbana) *y20* (Urbana) *k2* (Urbana)(T253)	Malate dehydrogenase null, yellow-green plant, tan saddle on seed coat	Hedges and Palmer (1992)
Mdh1-n (Columbia 1) *k2* (Columbia 1) (T261)	Malate dehydrogenase null, tan saddle on seed coat	Chen and Palmer (nd)
Mdh1-n (Ames 1) *y20* (Ames 1) (T317)	Malate dehydrogenase null, yellow-green plant	Amberger et al. (1992)
Mdh1-n (Ames 2) *y20* (Ames 2) (T323)	Malate dehydrogenase null, yellow-green plant	Hedges and Palmer (1992)
Mdh1-n (Ames 3) *y20* (Ames 3) (T324)	Malate dehydrogenase null, yellow-green plant	
Mdh1-n (Ames 4) *y20* (Ames 4) (T325)	Malate dehydrogenase null, yellow-green plant	
Mdh1-n (Ames 5)	Malate dehydrogenase null	Chen and Palmer (nd)
Mdh1-n (Ames 6)	Malate dehydrogenase null	
Mpi-a	Mannose-6-phosphate isomerase mobility variant	Chiang and Kiang (1988)

Table 5. Continued.

Gene	Phenotype	Reference
Mpi-b	Mannose-6-phosphate isomerase mobility variant	
Mpi-c	Mannose-6-phosphate isomerase mobility variant	
Mpi-d	Mannose-6-phosphate isomerase mobility variant	
Mpi-e	Mannose-6-phosphate isomerase mobility variant	Yu and Kiang (1993b)
mpi	Mannose-6-phosphate isomerase absent	Chiang and Kiang (1988)
Pi1	Protease inhibitor present	Kollipara et al. (1996)
pi1	Protease inhibitor absent in *Glycine tomentella*	
Pi2	Protease inhibitor present	
pi2	Protease inhibitor absent in *Glycine tomentella*	
Pi3	Protease inhibitor present	
pi3	Protease inhibitor absent in *Glycine tomentella*	
Pgi1-a	Phosphoglucose isomerase mobility variant	Chiang et al. (1987)
Pgi1-b	Phosphoglucose isomerase mobility variant	
pgi1	Phosphoglucose isomerase absent	
Pgi2	Phosphoglucose isomerase mobility variant	Chiang et al. (1987)
pgi2	Phosphoglucose isomerase absent	
Pgi3-a	Phosphoglucose isomerase mobility variant	Chiang et al. (1987)
Pgi3-b	Phosphoglucose isomerase mobility variant	

Table 5. Continued.

Gene	Phenotype	Reference
Pgm2-c	Phosphoglucomutase mobility variant	Yu and Kiang (1993b)
Pgm2-d	Phosphoglucomutase mobility variant	
Sdh - a	Shikimate dehydrogenase mobility variant	Yu and Kiang (1993b)
Sdh - b	Shikimate dehydrogenase mobility variant	
Sod2-a	Superoxide dismutase mobility variant	Griffin and Palmer (1989)
Sod2-b	Superoxide dismutase mobility variant	
sp1 (T293)	β -amylase null	Hildebrand and Hymowitz (1980); Gorman and Kiang (1978); Kiang (1981)
Sp1-c	β -amylase mobility variant	Griffin and Palmer (1986)

[a] References since 1986, except where previous references are necessary to describe the phenotype or the genetics of the trait.
[b] T-numbers are in the Genetic Type Collection.

Table 6. Genes affecting growth and morphology in soybean.

Gene	Phenotype	Reference
Br1 Br2 (T327)[a]	Branches originating from upper as well as lower nodes	Nelson (1996)
br1 br2 (T326)	Few branches originating from lower nodes	
Df6	Normal	Werner et al. (1987)
df6 (T286)	Dwarf	
Df7 or *Df8*	Normal	Foley et al. (1996)
df7 df8 (T281)	Dwarf	
dt1-t	Tall determinate stem	Thompson et al. (nd)
E5	Late flowering and maturity	McBlain and Bernard (1987)
e5	Early	
J	Normal	Ray et al. (1995)
j	Long juvenile trait	
Lmn	Normal	Yu and Kiang (1993a)
lmn	Leaf margin necrosis	
Lnr	Normal	Wilcox and Abney (1991)
lnr (T313)	Narrow rugose leaf	

[a] T-numbers are in the Genetic Type Collection.

Table 7. Genes affecting physiological traits in soybean.

Gene	Phenotype	Reference
Fg2-a	Normal kaempferol rutinoside	Buzzell and Buttery (1992)
Fg2-b	Less kaempferol rutinoside	
Fr5	Fluorescent root in UV light	Sawada and Palmer (1987)
fr5	Nonfluorescent	
Shr	Normal	Honeycutt et al. (1989)
shr (T311)[a]	Shriveled seed	

[a] T-numbers are in the Genetic Type Collection.

Table 8. Genes affecting fatty acid seed oil composition in soybean[a].

Gene	Phenotype	Reference
Fan	Normal	Rennie and Tanner (1989a,b)
fan (PI 123.440)	Reduced linolenic acid level	
fan (A5) (T307)[b]	Reduced linolenic acid level	
fan (C1640) (T280)	Reduced linolenic acid level	Wilcox and Cavins (1987)
fan (PI 361.088B)	Reduced linolenic acid level	Rennie et al. (1988); Rennie and Tanner (1989a)
fan2 (A23)	Reduced linolenic acid level	Fehr et al. (1992)
Fap1	Normal palmitic acid level	Erickson et al. (1988)
fap1 (C1726) (T308)	Reduced palmitic acid level	
fap2	Normal palmitic acid level	Erickson et al. (1988)
fap2 (C1727) (T309)	Increased palmitic acid level	
fap2-b	Reduced palmitic acid level	Fehr et al. (1991)
fap3	Reduced palmitic acid level	Schnebly et al. (1994)
fap4	Increased palmitic acid level	Schnebly et al. (1994)
Fas	Normal	Graef et al. (1985)
fas-a	Increased stearic acid level	
fas-b	Increased stearic acid level	
fas	Increased stearic acid level	

[a] References since 1986, except where previous references are necessary to describe the phenotype or the genetics of the trait.

[b] T-numbers are in the Genetic Type Collection.

Table 9. Genes affecting pest reaction in soybean[a].

Gene	Phenotype	Reference
Rbs1	Resistant to brown stem rot	Hanson et al. (1988)
rbs1	Susceptible	
Rbs2	Resistant to brown stem rot	Hanson et al. (1988)
rbs2	Susceptible	
Rbs3	Resistant to brown stem rot	Willmot and Nickell (1989)
rbs3	Susceptible	
Rcs3	Resistant to frogeye leaf spot races 2 and 5	Boerma and Phillips (1983); Phillips and Boerma (1982)
rcs3	Susceptible	
Rdc1	Resistant to stem canker	Kilen and Hartwig (1987)
rdc1	Susceptible	
Rdc2	Resistant to stem canker	Kilen and Hartwig (1987)
rdc2	Susceptible	
Rdc3	Resistant to stem canker	Bowers et al. (1993)
rdc3	Susceptible	
Rdc4	Resistant to stem canker	Bowers et al. (1993)
rdc4	Susceptible	
Rfs	Resistant to *Fusarium solani*	Stephens et al. (1993)
rfs	Susceptible	
Rmd-c	Resistant to powdery mildew	Lohnes and Bernard (1992)
rmd	Susceptible	
Rmi1	Resistant to Southern root-knot nematode	Luzzi et al. (1994)
rmi1	Susceptible	
Rpg2	Resistant to bacterial blight	Keen and Buzzell (1991)
rpg2	Susceptible	
Rpg3	Resistant to bacterial blight	Keen and Buzzell (1991)
rpg3	Susceptible	
Rpg4	Resistant to bacterial blight	Keen and Buzzell (1991)
rpg4	Susceptible	
Rpm2	Resistant to downy mildew race 2	Lim (1989)
rpm2	Susceptible	

Table 9. Continued.

Gene	Phenotype	Reference
Rps1-d	Resistant to *Phytophthora* races 1-7, 9-11, 13-16, 18, 21, 22, 24, 25	Buzzell and Anderson (1992)
Rps7	Resistant to *Phytophthora* races 12, 16, 18, 19	Anderson and Buzzell (1992)
rps7	Susceptible	
Rsv1	Resistant to soybean mosaic virus G1-6	Kiihl and Hartwig (1979); Roane et al. (1983)
Rsv1-k	Resistant to soybean mosaic virus G 1, 4	Chen et al. (1991)
Rsv1-m	Resistant to soybean mosaic virus G 1, 4, 5	
Rsv1-s	Resistant to soybean mosaic virus	Ma et al. (1995)
Rsv1-t	Resistant to soybean mosaic virus G 1, 2, 4-6	Kiihl and Hartwig (1979); Chen et al. (1991)
Rsv1-y	Resistant to soybean mosaic virus G 1-3	Chen et al. (1991)
rsv1	Susceptible	Kiihl and Hartwig (1979)
Rsv2	Resistant to soybean mosaic virus	Buzzell and Tu (1984)
rsv2	Susceptible (G 1-7)	
Rsv3	Stem-tip necrosis reaction to soybean mosaic virus	Tu and Buzzell (1987); Buzzell and Tu (1989)
rsv3	Mosaic response	

[a] References since 1986, except where previous references are necessary to describe the phenotype or the genetics of the trait.

Table 10. Cytoplasmic factors affecting chlorophyll deficiency in soybean.

Gene	Phenotype	Reference
cyt-Y4 (T314)[a]	Yellowish leaves	Cianzio and Palmer (1992)
cyt-Y5 (T315)	Greenish yellow leaves	
cyt-Y6 (T316)	Yellowish leaves, vigorous	
cyt-Y7 (T319)	Yellowish leaves, weak	
cyt-Y8 (T320)	Greenish yellow leaves	

[a] T-numbers are in the Genetic Type Collection.

Table 11. Nuclear genes affecting chlorophyll deficiency in soybean[a, b].

Gene	Phenotype	Reference
G2	Green seed coat	Reese and Boerma (1989)
g2	Yellow seed coat	
G3	Yellow seed coat	Reese and Boerma (1989)
g3 (T294)[c]	Green seed coat	
V2	Normal	Honeycutt et al. (1990)
v2 (T312)	Variegated leaf	
Y19	Normal	Palmer et al. (1990)
y19 (T265H)	Delayed albino	
Y20	Normal	Hedges and Palmer (1992)
y20 (Urbana) *k2* (Urbana) *Mdh 1-n* (Urbana) (T253)	Yellow-green plant, tan saddle on seed coat, malate dehydrogenase null	
y20 (Ames 1) *Mdh 1-n* (Ames 1) (T317)	Yellow-green plant, malate dehydrogenase null	Amberger et al. (1992)
y20 (Ames 2) *Mdh 1-n* (Ames 2) (T323)	Yellow-green plant, malate dehydrogenase null	Hedges and Palmer (1992)
y20 (Ames 3) *Mdh 1-n* (Ames 3) (T324)	Yellow-green plant, malate dehydrogenase null	
y20 (Ames 4) *Mdh 1-n* (Ames 4) (T325)	Yellow-green plant, malate dehydrogenase null	
Y21	Normal	Yee et al. (1986)
y21	Lethal yellow	
Y22	Normal	Palmer et al. (1990)
y22 (T270H)	Greenish yellow leaves	
Y23	Normal	Palmer et al. (1990)
y23 (T288)	Leaves becoming yellow-white and necrotic	

[a] References since 1986, except where previous references are necessary to describe the phenotype or the genetics of the trait.

[b] For T-strains (Genetic Type Collection) with an H suffix (eg., T265H), the allele is carried as the heterozygote because the homozygote is lethal, very weak, or sterile.

[c] T-numbers are in the Genetic Type Collection.

USDA-ARS AND UNIVERSITY OF ILLINOIS
National Soybean Research Laboratory
1101 West Peabody Drive
Urbana, Illinois 61801

1995-1996 Additions to the Isoline Collection of the USDA Soybean Genetic Collection

The Agricultural Research Service of the U.S. Department of Agriculture and the Illinois Agricultural Experiment Station, University of Illinois, have recently (in 1995 and 1996) released 69 near-isogenic lines (isolines) of soybeans described on the attached list. These lines were developed by backcrossing to the adapted commercial varieties Clark (maturity group IV, 9 lines), Harosoy (group II, 8 lines), and Williams (group III, 42 lines) or derivatives thereof such as L6 (Clark-*Rps1 rxp*) or Williams 82 (Williams-*Rps1-k*) as recurrent parents. Previous releases of isolines were made in 1972, 1975, and 1989 totaling 485 lines: 267 with Clark as the recurrent cultivar, 119 with Harosoy, 63 with Williams, and 36 with various other cultivars (2, 4). A complete list of these earlier releases is published in Soybean Genetics Newsletter 18:27-57, 1991 (4).

The isolines originated as F_2 or later generation plant selections from the indicated backcrosses (usually BC5) or combinations between such lines. They are homozygous for the indicated genes and have been selected for similarity to the recurrent parent for other traits. They involve over 50 different genes and a cytoplasmic factor. Closely linked genes are underlined. The attached list briefly describes the gene effects and lists published references. In the list of isolines, the recurrent parent is abbreviated: Wm for Williams, C for Clark, and H for Harosoy. L strains appearing in the parentage are followed by the transferred gene(s) and either the parentage is given or it may be found in SGN 18:27-57, 1991 (4).

The isoline development was conducted in the USDA-ARS soybean germplasm research program at the University of Illinois by R. L. Bernard and R. L. Nelson. Crop pollination and disease screening were done by ARS technician C. R. Cremeens. Composition analyses were done by T. Hymowitz, University of Illinois (for genes *Eu1-a, le, sp1,* and *Sp1-an*) and by ARS technician L. J. Bollinger (for genes *lx2, lx3, sun,* and *ti*).

Seeds of each line have been deposited in the National Plant Germplasm System and are available for research purposes, including development and commercialization of new cultivars, from the USDA Soybean Germplasm Collection, National Soybean Research Laboratory, 1101 West Peabody Drive, Urbana, Illinois 61801. It is requested that appropriate recognition be made if this germplasm contributes to the development of a new cultivar or when research is published using these isolines.

R. L. Bernard, University of Illinois
R. L. Nelson, USDA-ARS

CLARK ISOLINES

Transferred Gene(s)	Line	Parentage
		Disease Resistance
		Clark = E2 Rmd
rmd	L92-7229	C^6 x PI 317.334B, Kitamishiro
e2 rmd	L92-7259	L63-3117 $e2^6$ x PI 317.334B, Kitamishiro
		Stem Growth and Time of Maturity
		Clark = Dt1 e1 T E2 E3 E4 e5 L6 = Rps1 rxp from (C^8 x CNS) x (C^8 x Blackhawk)
dt1 E1 t e2 e3 Rps1 rxp	L82-2249	L70-4478 *dt1 E1 t e2 Rps1 rxp* x L71-920 *e2 e3*
dt1-t Rps1 rxp	L91-8052	L6 *Rps1 rxp*^{6} x Soysota
dt1-t Rps1 rxp	L91-8060	L6 *Rps1 rxp*^{6} x Peking
e4	L92-1166	C^6 x PI 297.550, Urosajnaja
E5	L92-1195	C x ancestor of L94-1110
e2 E5	L94-1110	L63-3117 $e2^6$ x L64-4830 (Harosoy^6 x PI 80.837)
		Chlorophyll
		Clark = g1 D1 D2 Y3 cyt-Y
d1	L74-854	L69-4663 *d1 d2* x L69-4659 *G1 d1*
G1	L76-1162	C x L69-4666 *G1 d2*
G1 d1 d2 cyt-G1	L93-2748	L62-1027 cyt-G1 x L64-2545 *G1 d1 d2*
d1 d2 y3	L93-2740	L69-4663 *d1 d2* x L63-2346 *y3*
		Pigmentation and Other Seed Traits
		Clark = b1 i-i K1 K2 R rps1 Rxp T W1
B1 e2	L93-2677	L63-3117 $e2^6$ x PI 65.388
i t-r w1	L81-5122	L70-4497 *i t* $w1^6$ x T136
R Rps1 rxp*	L72-1971	L67-3484 *i r Rps1* rxp^2 x (L65-1914*r Rps1* rxp^4 x T16)
i R Rps1 rxp*	L72-1950	L67-3484 *i r Rps1* rxp^2 x (L65-1914 *rRps1* rxp^4 x T16)
k1 k2	L92-9511	L67-3483 *k2* x L67-3479 *k1*
i k2	L92-9515	L67-3483 *k2* x L67-3469 *i*
i k2 r t	L94-1365	L67-3483 *k2* x L83-930 *i r t*

WILLIAMS ISOLINES

Transferred Gene(s)	Line	Parentage
	Disease Resistance	
Williams = E2 rpm1 rpm2 rps1 rps2 Rmd rj2 rps3 rps6 rps7 rsv1 Williams 82 (Wm 82) = *Rps1-k* from Wm^7 x Kingwa		
rmd	L90-7978	Wm^6 x Jefferson
rmd	L94-2018	$Williams^6$ x Harosoy
Rpm2	L87-0174	Wm^6 x PI 88.788
Rps1	L88-8470	Wm^2 x Union, BC4 Wm with *Rps1* from Mukden
Rps1-d	L93-3312	Wm^6 x PI 103.091, Wu An
Rps3-b	L89-1541	Wm^6 x PI 82.312N
e2 Rps3-b	L89-1550	Wm^6 x PI 82.312N
Rps3-b	L91-8347	Wm^6 x PI 172.901
Rps3-c	L92-7857	Wm^6 x PI 340.046
Rps6	L89-1581	Wm^6 x Altona
Rps7	L93-3258	Wm^6 x Harosoy
Rsv1-m	L84-2112	Wm x ($Will^6$ x Marshall)
e2 Rsv1-m	L84-2157	Wm x ($Will^6$ x Marshall *e2 Rsv1-m*)
rsv1-t	L93-3327	Wm^6 x Ogden
Rsv1-?	L92-8580	Wm^6 x PI 483.084, Suweon 97
rmd Rps3	L92-7963	Wm^6 x PI 86.972-1
Rpm1 Rps1 Rps2 Rmd-c Rj2	L82-1657	L75-6141 *Rpm1 Rps1* x L76-1988 *Rps2 Rmd-c Rj2*
Rpm1 Rps1 Rps2 Rmd-c Rj2 Rsv l	L83-462	(L78-379 *Rsv1* x Wm 82) x (L75-6141 x L76-1988)
Crossovers:		
Rps2 rmd rj2	L91-8915	L76-1988 *Rps2 Rmd-c Rj2* x L82-2024 *rmd Ti-b*
rps2 rmd Rj2	L91-8765	L76-1988 *Rps2 Rmd-c Rj2* x L82-2024 *rmd Ti-b*
rps2 Rmd-c Rj2	L91-8839	L76-1988 *Rps2 Rmd-c Rj2* x L82-2024 *rmd Ti-b*

Stem Growth and Time of Maturity

Williams = dt2 E2 rps1 rsv1 Will = Dt2 from Wm[6] x T117

e2	L88-8153	Wm[6] x Altona
e2	L89-1553	Wm[6] x PI 82.312N
e2	L92-7647	Wm[6] x L63-3117 (Clark[6] x PI 86.024 *e2*)
e2 Rps1-k	L92-7677	Wm 82[6] x L63-3117 (Clark[6] x PI 86.024 *e2*)
Dt2 Rsv1-m	L82-951	Will[6] x Marshall *Rsv1-m*
Dt2 e2 Rsv1-m	L88-8629	Will[6] x Marshall *e2 Rsv1-m*

Transferred Gene(s)	Line	Parentage

Pigmentation and Seed Composition

Williams = Eu1-b g1 g2 i-i Le Lx2 Lx3 R rj2 Rmd rps1 rps2 rsv1 Sp1-b Sun T Ti-a w1

Eu1-a Rps1-k	L91-8520	Wm 82[6] x Columbia
G1 Rps1-k	L93-3423	Wm 82[6] x Columbia
G2	L93-3103	Wm[6] x Ogden
I	L89-2435	Wm[6] x (Harosoy[5] x D54-2437)
I r Rsv1-?	L93-7333	L76-1994 *I Rps2 Rmd-c Rj2* x L88-8440 *r Rsv1-?*
I r Rsv1-? Rps2 Rmd-c Rj2	L93-3539	L76-1994 *I Rps2 Rmd-c Rj2* x L88-8440 *r Rsv1-?*
I t Rps2 Rmd-c Rj2	L94-2285	L76-2023 *t Rps2 Rmd-c Rj2* x L76-1994 *I Rps2 Rmd-c Rj2*
le Rps1-k	L90-8047	Wm 82[6] x Wilson Five
lx2 Rps1-k	L94-2576	Williams 82[6] x Century L2-3 (Century[4] x PI 86.023)
lx3 Rps1-k	L93-7290	Wm 82[6] x PI 417.458, Wase Natsu
sp1 Rps1-k	L90-8003	Wm 82[6] x T293, Altona
Sp1-an Rps1-k	L89-2621	Wm 82[6] x Chestnut
i Sp1-an Rps1-k	L89-2634	Wm 82[6] x Chestnut
sun ti Rps1-k	L91-8558	Kunitz (Wm 82[6] x PI 157.440, Kum Du) x L85-2196 (Wm[6] x PI 229.324, Itachi)
W1	L92-7600	Wm[6] x Clark

HAROSOY ISOLINES

Harosoy = g1 D1 D2 Dt1 dt2 e1 t E3 lf1 Ln rps2 rmd rj2 s

dt2 E1 T e3	L74-102	[(H^6 x T204 *e3*) x (H^6 x PI 196.166 *E1 T*)] x [(H^6 x PI 196.166 *E1 T*) x (H^6 x Higan dt_1)]
Dt2 Lf1	L64-1067	(H^6 x T117 *Dt2*) x (H^6 x PI 86.024 *Lf1*)
Dt2 ln	L64-1061	(H^6 x T117 *Dt2*) x (H^6 x T204 *ln*)
G1	L90-7656	H^6 x PI 81.763
G1 d1 d2 E1	L64-2511	H^6 x Columbia
s-t	L93-2589	H^6 x L67-3243 *e2 s-t* from (C^6 x PI 86.024 *e2*) x (C^6 x Chief *s-t*)

Crossovers:

Rps2 Rmd-c rj2	L90-4683	H^6 x D54-2437
rps2 Rmd-c Rj2	L90-4711	H^6 x D54-2437

L82-1449, previously released from this cross, is *Rps2 Rmd-c Rj2*
D54-2437 parentage is CNS, Lincoln, Ogden, Richland, and Roanoke with linked genes *Rps2 Rmd-c Rj2* from CNS.

Original Allele	Transferred Gene	Reference	Effect of Transferred Gene
b1	*B1*	14, 15	bloom on seed coat (in combination with *B2 B3* already present in Clark)
cyt-Y1	*cyt-G1*	14	stay-green, plant & seeds do not turn yellow
D1 D2	*d1 d2*	14, 15	stay-green, similar to cyt-G but deeper green (*d1 D2* has very light green seeds)
Dt1	*dt1*	14, 15	determinate stem
Dt1	*dt1-t*	19	tall determinate stem
dt2	*Dt2*	14, 15	semi-determinate stem
e1	*E1*	14, 15	late maturity
E2	*e2*	14, 15	early maturity (Harosoy has *e2* allele)
E3	*e3*	14, 15	early maturity
E4	*e4*	14, 15	early maturity, less photoperiod sensitivity
e5	*E5*	13	late maturity
Eu1-b	*Eu1-a*	8, 14, 15	urease slow band
g1	*G1*	14, 15	green seed coat
g2	*G2*	17	green seed coat
i-i	*i*	14, 15	dark-pigmented seed coat
i-i	*I*	14, 15	light-pigmented seed coat and hilum

K1	*k1*	14, 15	saddle pattern on seed coat
K2	*k2*	14, 15	tan saddle on seed coat
Le	*le*	14, 15	seed lectin null
lf1	*Lf1*	14, 15	5 leaflets
Ln	*ln*	14, 15	narrow leaflet
Lx2	*lx2*	14, 15	lipoxygenase-2 null
Lx3	*lx3*	14, 15	lipoxygenase-3 null
R	*r*	14, 15	brown seed pigment (Harosoy has *r* allele)
R	*R**	6	brown hilum with black seed coat (mutable)
rj2	*Rj2*	14, 15	ineffective nodulation to some races
Rmd	*rmd*	14, 15	powdery mildew susceptible (Harosoy has *rmd*)
Rmd	*Rmd-c*	12	resistant to powdery mildew (adult and juvenile)
rpm1	*Rpm1*	14, 15	resistant to downy mildew
rpm2	*Rpm2*	11	resistant to downy mildew
rps1	*Rps1*	14, 15	resistant to Phytophthora
"	*Rps1-d*	5	" " "
"	*Rps1-k*	14, 15	" " "
rps2	*Rps2*	14, 15	" " "
rps3	*Rps3*	14, 15	" " "
"	*Rps3-b*	16	" " "
"	*Rps3-c*	3	" " "
rps6	*Rps6*	14, 15	" " "
rps7	*Rps7*	1	" " "

Original Allele	Transferred Gene	Reference	Effect of Transferred Gene
rsv1	*Rsv1*	14, 15	resistant to soybean mosaic virus
rsv1	*Rsv1-m*	7	" " " " "
rsv1	*rsv1-t*	14, 15	" " " " "
rsv1	*Rsv1-?*	10, Unpub.	" " " " "
Rxp	*rxp*	14, 15	resistant to bacterial pustule
s	*s-t*	14, 15	long internode
Sp1-b	*Sp1-an*	14, 15	ß-amylase activity weak or absent, seed protein band present
Sp1-b	*sp1*	14, 15	ß-amylase null
Sun	*sun*	9	seed urease null
T	*t*	14, 15	gray pubescence (Harosoy has *t* allele)
T	*t-r*	18	gray pubescence with red-buff seed coat
Ti	*ti*	14, 15	Kunitz trypsin inhibitor null
W1	*w1*	14, 15	white flower (Williams has *w1* allele)
Y3	*y3*	14, 15	chlorotic plant, hypostatic to *G1*

REFERENCES

1. Anderson, T. R., and R. I. Buzzell. 1992. Inheritance and linkage of the Rps7 gene for resistance to Phytophothora rot of soybean. Plant Disease 76:958-959.

2. Anon. 1974. Notice of release of isolines of Clark and Harosoy soybeans. SGN 1:66-74.

3. Athow, K. L., F. A. Laviolette, A. C. L. Hahn, and L. D. Ploper. 1986. Genes for resistance to Phytophthora megasperma f.sp. glycinea in PI 2734838D, PI 64747, PI 274212, PI 82312N, and PI 3400046. SGN 13:119-131.

4. Bernard, R. L., R. L. Nelson, and C. R. Cremeens. 1991. USDA Soybean Genetic Collection: Isoline Collection. SGN 18:27-57.

5. Buzzell, R. I., and T. R. Anderson. 1992. Inheritance and race reaction of a new soybean Rps1 allele. Plant Disease 76:600-601.

6. Chandlee, J. M., and L. O. Vodkin. 1989. Unstable expression of a soybean gene during seed coat development. Theor. Appl. Genet. 77:587-594.

7. Chen, P., G. R. Buss, C. W. Roane, and S. A. Tolin. 1991. Allelism among genes for resistance to soybean mosaic virus in strain-differential soybean cultivars. Crop Sci. 31:305-309.

8. Kloth, R. H., and T. Hymowitz. 1985. Re-evaluation of the inheritance of urease in soybean seed. Crop Sci. 25:352-354.

9. Kloth, R. H., J. C. Polacco, and T. Hymowitz. 1987. The inheritance of a urease-null trait in soybeans. Theor. Appl. Genetics 73:410-418.

10. Lim, S. M. 1985. Resistance to soybean mosaic virus in soybeans. Phytopathology 75:199-201.

11. Lim, S. M. 1989. Inheritance of resistance to Peronospora manshurica races 2 and 33 in soybean. Phytopathology 79:877-879.

12. Lohnes, D. G., and R. L. Bernard. 1992. Inheritance of resistance to powdery mildew in soybeans. Plant Disease 76:964-965.

13. McBlain, B. A., and R. L. Bernard. 1987. A new gene affecting the time of flowering and maturity in soybeans. J. Heredity 78:160-162.

14. Palmer, R. G., and T. C. Kilen. 1987. Qualitative genetics and cytogenetics, Chapter 4 in J. R. Wilcox, ed. Soybeans: Improvement, Production, and Uses. 2nd ed. pp. 139-209. American Society of Agronomy.

15. Palmer, R. G., R. C. Shoemaker, and B. Rennie. 1987. Approved soybean gene symbols. SGN 14:41-58.

16. Ploper, L. D., K. L. Athow, and F. A. Laviolette. 1985. A new allele at the Rps3 locus for Resistance to Phytophothora megasperma f.sp. glycinea in soybean. Phytopathology 75:690-694.

17. Reese, P. F., and H. R. Boerma. 1989. Additional genes for green seed trait in soybean. J. Hered. 80:86-88.

18. Seo, Y. W., J. E. Specht, G. L. Graef, and R. A. Graybosch. 1993. Inheritance of red-buff seed coat in soybean. Crop Sci. 33:754-758.

19. Thompson, J. A., R. L. Bernard, and R. L. Nelson. 1994. A third allele at the dt1 locus. SGN 21:257-260. (also submitted to Crop Science)

IOWA STATE UNIVERSITY
USDA/ARS Field Crops Research
Department of Agronomy
Ames, IA 50011

SOYBASE, A SOYBEAN GENOME DATABASE

SoyBase is an ACE-type database with hypertext links between classes. SoyBase combines data from many laboratories, the literature and other databases (GRIN, PVP, AGRICOLA, BIOSIS, Genbank) into a central location. Information is displayed via an easy to learn graphical interface where the user clicks on items to open windows which contain the requested information.

SoyBase can be accessed via the WWW at http://macgrant.agron.iastate.edu. This page offers an easy way for users to provide data to be included in SoyBase and also has links to other soybean and legume information available on the Web. In addition to the WWW version, the entire database is available in a Macintosh version. Contact David Grant (dgrant@iastate.edu or from the SoyBase home page) for availability and hardware requirements.

SoyBase currently includes several types of soybean data which are summarized in the following table and discussed in more detail afterwards.

Data Class	Entries	Short Description
Map_Collection	6	Classical genetic map and 5 molecular marker maps
Locus	563	Morphological, biochemical and molecular markers
Probe	358	DNA markers with images of the screening autoradiograms for most of the core set of markers developed at ISU
Gene	370	Data include alleles, map location and 2 point segregation data
QTL_Study	33	Data on 21 agronomic traits including yield, seed quality and disease resistance
Germplasm	>30,000	Soybean germplasm with pointers to GRIN and PVP databases and hypertext links to the enzyme, storage protein, nodulin and allele classes
Traits	234	Trait descriptors associated with entries in the GRIN and PVP databases
Reaction_or_Pathway	41	Clickable diagrams of 315 metabolic pathways covering 524 enzymes and 444 metabolites
Enzyme	524	Data include EC number, purification, clones, physical properties and the species and cultivars studied
Storage_Protein	14	Data on 6 vegetative and 8 seed storage proteins

Nodulin	37	Data on the nodulins of soybean including gene location and structure and physical properties and function of the gene product
Sequence	580	All available Glycine DNA sequences with pointers to the Genbank records
Pathology	15	Information on soybean diseases including causative organism, differentials, phenotypic scores and distribution
Author	>22,000	Contains the names of all of the authors of the >18,000 AGRICOLA, BIOSIS and direct entry papers related to soybean
Colleague	>250	Names and address of soybean-associated people

Genetic Maps

The classical gene map contains 29 linkage groups, and the underlying 2 point linkage data are in the database. The five molecular maps represent independent mapping populations with significant overlap between the markers used. For many of the RFLP markers images of autoradiograms are available which indicate the exact DNA fragment that was used to place an RFLP marker on a map. Related information about genes, alleles, and molecular probes is available via hypertext links from the map diagrams.

QTL Studies

For each QTL study there are data on the trait evaluated, the population used and the associations between the phenotypic traits and the molecular markers. A hypertext link is available between these linked loci and the genetic map diagrams.

Germplasm

SoyBase contains approximately 30,000 germplasm records with pedigree information and trait data. Direct links to the PVP and GRIN databases are provided as well as hypertext links to related data within SoyBase.

Metabolic Pathways and Enzymes

There are diagrams covering 41 pathways for nitrogen and fatty acid synthesis and degradation. For each pathway, information is available for the enzymes, reactants, products, and cofactors involved along with the data on the kinetics, inhibition, activation, and regulation of the enzymatic steps. More than 500 individual enzymes are detailed.

Storage Proteins

These records contain physical and genetic data on all of the characterized seed and vegetative storage proteins of soybean.

Nodulins

SoyBase currently includes information on 37 nodulins. Examples of the data available are the nature of the gene product encoded by the nodulin gene and its function, the host and inducing species, information on the genetics and genome organization of the gene and availability of DNA and antibody probes.

Sequences

Direct links are provided to the Genbank records for all Glycine DNA and protein sequences.

Pathology and Diseases

Fifteen soybean diseases are covered in SoyBase, including data on the causative organism, differentials and phenotypic scores, and the genetics of resistance.

Authors and References

All references in AGRICOLA that are related to soybean genetics are incorporated into the database. Recent references from BIOSIS are also available while those prior to 1995 will be added in the near future. Other peripherally related references such as those for heterologous probes or metabolic data from other species are also included.

Colleagues

SoyBase currently contains entries for more than 250 persons who are involved with soybean research. This list is certainly not complete. Please use the form on the SoyBase home page (see above) to provide corrections and/or additions to the list.

David Grant
Marcia Imsande
Randy Shoemaker

UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE
Campos, RJ
Brazil

USDA-ARS
Iowa State University
Ames, Iowa USA

Pollen germination studies

Pollen germination tests have been reported to provide reasonable estimates of pollen fertility (Brewbaker and Kwack, 1963). Our objective was to test pollen germination of four PS (partial sterile) mutants and to make comparisons with the results of pollen germination of fertile plants. The four PS mutants were recovered from a gene tagging study (Palmer et al., 1989). The mutants are male fertile, as judged by pollen staining and fluorescence (Pereira, 1994). PS-1 is inherited as a single recessive gene, and PS-2, PS-3, and PS-4 are maintained as heterozygotes and upon self-pollination segregate 1 fertile: 1 partial sterile plant. All four mutants are partial female sterile.

Plants were grown in a growth chamber. The day temperature was 29°C and the night temperature 26° C. The photoperiod was 18 hours the first four weeks, 16 hours for one week, and then continuous 14 hours.

A factorial boric acid x sucrose combination was tested (Table 1). The most consistent results were obtained with 10% sucrose and 30 ppm boric acid.

Freshly opened flowers were collected early in the morning. Pollen grains from individual flowers were sprinkled onto the drop of sucrose x boric acid solutions on each slide and allowed to grow at room temperature. After 60 minutes, the germinated pollen grains, nongerminated pollen grains, and burst pollen grains were counted. A pollen grain was considered germinated if the pollen tube had attained a length of at least 4 times the pollen grain diameter (30 mm). Data are recorded as the percentage of pollen grains germinated in one observed microscope field (125 x magnification).

The pollen germination tests indicated that the pollen grains of the four PS mutants were able to germinate as well as pollen grains from fertile plants (Tables 2 and 3). In paraffin serial sections of ovules one day after anthesis, we observed traces of pollen tubes entering into the micropyle. We believe that the pollen grains of the four PS mutants are able to effect fertilization.

References

Brewbaker, J. L. and B. H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen-tube growth. American Journal of Botany 50:859-865.

Palmer, R. G., B. R. Hedges, R. S. Benavente, and R. W. Groose. 1989. The w4-mutable line in soybean. Developmental Genetics 10:542-551.

Pereira, T. N. S. 1994. Genetic and cytological analyses of four partial-sterile mutants in soybean (Glycine max L. Merr.). Ph. D. dissertation. Iowa State University, Ames.

T. N. S. Pereira
R. G. Palmer USDA

Table 1. Percentage pollen germination from PS-1 mutant in sucrose x boric acid solutions.

Sucrose (%)	Boric acid (ppm)	Germination (%)
0	0.0	0.0
5	0.0	0.0
10	0.0	24.5
0	7.5	74.0
5	7.5	69.0
10	7.5	60.0
0	15.0	47.5
5	15.0	47.5
10	15.0	70.5
0	30.0	37.5
5	30.0	45.0
10	30.0	87.5

Table 2. Percentage pollen germination from normal plants and PS-1 mutant plants in 10% sucrose and 30 ppm boric acid solution.

Plant	Normal	PS-1
1	90.0	93.0
2	89.5	84.5
3	92.0	86.0
4	88.5	83.5
5	85.0	92.0
Average	89.0	87.8

Table 3. Percentage pollen germination from normal plants and partial-sterile mutants (PS-2, PS-3, and PS-4) in two combinations of sucrose x boric acid solutions.

Plant	5% Sucrose and 7.5 ppm boric acid	10% Sucrose and 30 ppm boric acid
Normal	89	86
PS-2	87	83
Normal	83	80
PS-3	81	83
Normal	86	87
PS-4	88	86

AGRICULTURE AND AGRI-FOOD CANADA
Plant Research Centre
Ottawa, Ontario, CANADA K1A 0C6

E3 and *Dt1* Linkage

Buzzell and Palmer (1989) reported that the E3 and Dt1 loci did not segregate independently but they did not estimate linkage. The E3 allele results in later flowering and maturity and sensitivity to natural daylength extended to 20 h with cool-white fluorescent lamps (Buzzell, 1971), while the e3 allele results in early maturity and photoperiod insensitivity. The E3 and E4 loci control sensitivity to natural daylength extended to 20 h with incandescent lamps (Saindon et al., 1989). The Dt1 loci affects stem termination (Bernard, 1972).

From a cross, using Harosoy near-isogenic lines, of OT89-5 (PI 546.043; e3 e4 Dt1) x OT94-39 (PI 591.434; E3 e4 dt1), F2 plants were evaluated under natural daylength extended to 20 h with incandescent lamps for photoperiod sensitivity (incandescent long daylength) and stem termination type. Both parental genotypes were e4e4 which facilitated evaluation of the E3 locus under incandescent long daylength. In an e4e4 background, the E3 allele resulted in a flowering delay of about 30 d and plants failed to reach maturity before frost under incandescent long daylength, while the e3 allele was insensitive to incandescent long daylength (Cober et al., 1996). E3 plants reached approximately R6 at first frost (29 September 1995) while e3 plants matured about 15 September 1995 in this study.

Single locus analysis showed that the observed 624 photoperiod-sensitive : 203 photoperiod-insensitive fit a 3:1 ratio (P=0.794) for the E3 locus. The observed 604 indeterminate : 223 determinate plants fit a 3:1 ratio (P=0.206) for the Dt1 locus. Results for 827 F2 plants were: 415 E3 Dt1, 209 E3 dt1, 189 e3 Dt1, 14 e3 dt1 which did not fit a 9:3:3:1 ratio (P<0.0001) indicating that E3 and Dt1 are linked. LINKAGE-1 (Suiter et al., 1983), which uses the method of maximum likelihood, estimated a linkage of 27.52 ± 3.23 cM. Dt1 is in linkage group V with L1 (Weiss, 1970) and the linkage of Dt1 and E3 extends this linkage group. E3 is also linked to Rmd, a locus conferring resistance to powdery mildew (Microsphaera diffusa Cke & Pk)(Buzzell and Palmer, 1989) but Dt1 and Rmd have not been tested.

REFERENCES

Bernard, R. L. 1972. Two genes affecting stem termination in soybeans. Crop Sci. 12:235-239.

Buzzell, R. I. 1971. Inheritance of a soybean flowering response to fluorescent-daylength conditions. Can. J. Genet. Cytol. 13:703-707.

Buzzell, R. I., and R. G. Palmer. 1989. Rmd and E3 linkage. Soybean Genet. Newsl. 16:29-30.

Cober, E. R., J. W. Tanner, and H. D. Voldeng. 1996. Genetic control of photoperiod response in early-maturing soybean near-isogenic lines. Crop Sci. (in press).

Saindon, G., H. D. Voldeng, W. D. Beversdorf, and R. I. Buzzell. 1989. Genetic control of long daylength response in soybean. Crop Sci. 29:1436-1439.

Suiter, K. A, J. F. Wendel, and J. S. Case. 1983. LINKAGE-1: a PASCAL computer program for the detection and analysis of genetic linkage. J. Hered. 74:203-204.

Weiss, M. G. 1970. Genetic linkage in soybeans: Linkage group V and VI. Crop Sci. 10:469-470.

Elroy R. Cober

Harvey D. Voldeng

SOYBEAN RESEARCH INSTITUTE
Nanjing Agricultural University
Nanjing, Jiangsu 210095
The People's Republic of China

A Comparison of Morpho-Genesis Ability of Different Callus Types of Soybeans

It is of importance for improving frequency of plant regeneration in tissue culture to classify, identify and regulate the morphotypes of callus. In wheat protoplast culture, to select suitable morphotypes of callus at various stages has been recognized as a key point of successful plant regeneration. The frequency of plant regeneration of soybean tissue culture was low, and even lower than other major field crops. The objective of this paper was to classify the morphotypes of callus in soybean tissue culture and to investigate the morpho-genesis ability of the different morphological callus types, or in other words, the potential ability of plant regeneration of the different callus types.

Materials and Methods

It was thought that the seeds and young seedlings were most convenient sources of explants. The cultivar "Hongyin No. 1" was used and explants were taken from the 5-6 days old seedlings. The basal medium was 1/2MS (Murashige and Skoog, 1962) plus 500 mg/L CH (casein hydrolysate), 3% sucrose, and 0.8% gelrite, and was adjusted to pH 6.0 before autoclaving 15 minutes at 120°C, 120 kpa. Explants obtained from seedlings were cut into 1-2 mm or $2x2mm^2$. After 14-16 days culture on induction medium, a number of calli were produced. The induction medium was the basal medium plus 5 mg/L BA, 2 mg/L IAA and 2 mg/L KT. After about 20 days culturing on the induction medium, the formed callus were selected and transferred onto secondary medium which was the induction medium plus 1 mg/L GA_3 or 1.5 mg/L ABA. Later on, the calli were transferred onto differentiation medium. The bud differentiation medium was the basal medium plus 1.2 mg/L BA or 1 mg/L IAA + 1mg/L KT; the root differentiation medium was the basal medium plus 0.5 mg/L IBA or 0.5 mg/L IAA; the embryoid differentiation medium was the basal medium plus 0.5 mg/L KT + 0.5 mg/L BA or 2 mg/L 2,4-D + 0.01 mg/L KT. The calli were subcultured one to two times each for two weeks, and examination of fresh weight, color of somatic embryos and the number of regenerated plantlets were taken.

Results: Morphotypes of callus

It was found that the explants from different parts of seedlings produced different frequencies of callus induction and different growth rates of callus, but the former was all over 84%. Four types of callus were observed and classified as: the light green, compact and tubercular callus (type A); the

green white, compact and tumorous callus (type B); the light yellow, loose friable and granular callus (type C); and the yellow brown, relatively loose and round tubercular callus (type D) (Table 1).

Morphogenesis and organogenesis

The type A callus could differentiate a large number of roots and buds and form plantlet but embryogenesis frequency was relatively low (Table 2). Therefore, type A callus could be considered as a kind of organogenetic callus. The explants from mature cotyledon mostly produced type A callus. The type B callus only formed many roots, but few buds and somatic embryos under suitable conditions. The type C callus could differentiate a large number of somatic embryos after transferred onto differentiation medium and regenerate a whole plantlet, so it was considered as a kind of embryogenetic callus. The type D callus divided vigorously in early subculture stage and stopped growing gradually in late stage.

Embryogenesis

The callus type C were transferred onto embryoid differentiation medium and the frequency of embryoid could be up to 44.6%. The type C callus could come from a wide range of explants, such as leaf, hypocotyl, epicotyl, and cotyledon of young seedlings, in other words, the explants from all parts of young seedlings had the ability of embryogenesis, but the frequency of cotyledon was higher than that of the others.

Effect of GA_3 and ABA

The different types of callus were GA_3 (1 mg/L) and ABA (1.5 mg/L), respectively. It was found that the organogenesis and embryogenesis ability varied greatly (Table 3). In the medium with GA_3, the frequency organogenesis (buds and roots), was increased, but the frequency of embryogenesis was decreased. But in the medium with ABA, both organogenesis and embryogenesis ability could be improved.

Early identification of morphotypes of callus for improving differentiation frequency

Among the four types of callus in soybean somatic cell culture, the type A was favorable to organogenesis, which differentiated a large number of roots and buds; the type C was favorable to embryogenesis; the type B could become A or C under given conditions (adding GA_3 or ABA to subculture medium); the type D was inferior for both organogenesis and embryogenesis. Accordingly, after one to two times of subculture of the young callus, to pick up type A, B, and C callus and discard type D callus for further culture can improve the differentiation frequency of callus. Because the type B callus is changeable, regulation can be made by adding GA_3 to the subculture medium to change B type callus into A type callus and adding ABA to change B into type C callus. GA_3 could improve the frequency of bud and root differentiation for all types of callus, but inhibit the embryogenesis. In contrary, ABA could improve embryogenesis and plant regeneration for all types of callus.

References

Chen yuanling et al. 1994. Studies on embryogenetic morphotypes identification in rice. Journal of South China Agricultural University. 15(1):78-84.

Heyser, J. W. et al. 1983. High frequency, long term regeneration of rice from callus culture. Plant Science Lett. 29:175-182.

Trolinder, N. L. et al. 1988. Somatic embryogenesis in cotton (*Gossypium*). II. Requirement for embryo development and plant regeneration. Plant Cell, Tiss. Org. Cult. 12:43-53.

Mei Chuansheng et al. 1994. Regulation of ABA on plant regeneration frequency in vitro in rice.Journal of Agrobiotechnology. 2(1):96-98.

Junyi Gai

Zibiao Guo

Table 1. Comparison of morphological types of soybean calli.

Type	Shape	Surface	Color	Texture	Source of explant
A	Tubercular	Smooth	Light green	Compact, hard	Cotyledon, hypocotyl, young leaves
B	Tumorous	Relatively smooth	Green white	Compact, hard	Hypocotyl, young leaves
C	Granular	Rough	Light yellow	Loose, friable	Cotyledon, hypocotyl, epicotyl, young leaves
D	Round tubercular	Irregular	Yellow brown	Relatively loose, soft	Hypocotyl, cotyledon

Table 2. Comparison of morpho-genetic ability among callus types.

Callus type	No. inoculum	Differentiation of bud (%)		Differentiation of root (%)		Differentiation of embryoid (%)	
		BA1.3	BA1+KT1	IBA0.5	IAA0.5	2,4-D2+KT0.01	KT0.5+BA0.5
A	144 X 3	13.0	30.2	90.0	88.7	2.1	1.2
B	158 x 4	2.3	1.7	81.0	67.1	0.3	0.6
C	146 X 5	0.7	0.3	59.8	48.6	44.6	13.2
D	235 X3	0	0	2.3	1.4	0	0

Table 3. Effect of GA_3 and ABA on morphogenetic capacity of soybean calli.

Regulator	Callus type	No. inoculum	Frequency of bud differentiation (%)		Frequency of root differentiation (%)		Frequency of embryoid differentiation (%)	
			BA1.2	BA1+IAA1	IBA0.5	IAA0.5	2, 4-D1+KT0.01	KT0.5+BA0.5
	A	50 X 3	22.3	35.1	95.0	91.7	1.1	0.6
GA_3	B	50 X 3	3.8	2.6	85.0	71.0	0	0.3
	C	50 X 3	0.8	0.4	71.0	67.5	21.1	7.7
	D	50 X 3	0.8	0.1	18.0	14.0	0	0
	A	50 X 3	21.2	31.2	71.0	90.1	8.7	4.3
ABA	B	50 X 3	3.0	2.6	82.5	78.4	7.3	10.1
	C	50 X 3	0	0.4	6.3	58.8	4.6	43.8
	D	50 X 3	0.1	0.1	3.8	2.9	3.0	4.2

SOYBEAN RESEARCH INSTITUTE
Nanjing Agricultural University
Nanjing, Jiangsu 210095
The People's Republic of China

A Microanalytical Method of Tofu Yield

Introduction

To determine the output of tofu in the study of its inheritance, two kinds of generation, plant generation and seed (or exactly embryo) generation could be used. Seeds of a F_1 plant could be looked as F_1 generation on plant generation basis, but an embryo (or roughly a seed) generation basis. Therefore, a microanalytical method on single or a few seeds basis is needed for the study of inheritance of tofu yield and quality. Breeding for tofu yield and quality in a scientific program has been getting important in China and east Asia (Wang, 1983) The present paper deals with such kind of microanalytical technique of tofu yield with comparison to the standard method of large specimen.

Materials and Methods

Three soybean varieties with different 100-seed weight were used to study the microanalytical method. They were N21543, Nannong 88 - 48, and Tongzhoudou with different seed size (100 - seed weight being 13. lg, 23.3g, and 28.0g, respectively). The treatments of specimen size were set as one seed, two seeds, four seeds, six seeds, eight seeds and ten seeds with standard method (using 50g dried seeds) as check.

Ten replications were used for each sample size. In microanalytical method, soybean seeds were soaked in 5 ml distilled water overnight (12 -24 hours) at 20°C. The soaked soybeans were ground in mortar and centrifugalized under 3000 rpm for 15 min. to remove the sediment. The final volume of soymilk was adjusted to 6 mL with distilled water. A total of 6 ml fresh soymilk was heated on a hot plate to boiling with constant stirring and then kept in 80°C waterbath. The coagulant, calcium sulfate, was added into the hot soymilk according to soybean seed weight (Lim, et al. 1990). After setting for 20 min., the curds were centrifugalized under 2000 rpm for 10 min. to achieve a compressing action. The weight of fresh tofu was recorded. After tofu was dried in 105°C for five hours, the weight of dry tofu was obtained. The standard method was in accordance with the method of Zhou et al. (1992).

Results and Discussion

Table 1 showed that there were no significant variation found among specimen sizes in both fresh tofu yield and dried tofu yield per gram sobean seeds, but there existed significant variation among three soybean varieties.

Table 1. Analysis of variance of fresh and dried tofu yield among various specimen sizes

Trait	Source of variation	DF	SS	MS	F
Fresh Tofu Yield	Specimen size	6	0.201	0.0335	1.106
	Variety	2	5.432	2.716	89.64**
	Size X Variety	12	0.191	0.0159	0.525
	Error	189	5.731	0.0303	
Dried Tofu Yield	Specimen size	6	0.003	0.005	0.69
	Variety	2	0.038	0.019	263.89**
	Size X Variety	12	0.007	5.8×10^{-4}	0.806
	Error	189	0.136	7.2×10^{-4}	

Note: ** represents significant at the 0.01 level.

The t - tests between the means of various specimen sizes and those of the standard method were all not significant for both fresh tofu and dried tofu yield on per gram soybean seed basis for both individual varieties and the pooled data, which indicated that the microanalytical method with various specimen sizes could provide a same result of the estimate of population mean as the standard method did (Table 2,3). Meanwhile, the F - tests between the mean squares of specimen sizes and those of the standard method were all not significant for both fresh tofu and dried tofu with the exception of 4 - seeds and 10 - seeds specimen of pooled data for fresh tofu yield and 1 - seed specimen of Tongzhoudou, Nannong 88 - 48 and pooled data for dried tofu yield, which suggested that sampling errors of all kinds of specimen sizes were not significantly larger than those of the standard method except the cases stated above. The sampling fluctuation of both micro - specimen and standard specimen for fresh tofu yield was larger than that for dried tofu according to the c.v. values in Table 2, 3. It might be due to that the water content of fresh tofu varied seriously and was not stable among measurements. Therefore, in determination of specimen size, the sampling error of dried tofu yield rather than fresh tofu yield should be considered as the major factor. The c. v. values in Table 2. and Table 3. also showed that the sampling fluctuation of micro specimen was larger than that of standard specimen for fresh tofu yield, but for dried tofu yield they were about the same except that of single seed specimen. Accordingly, the microanalytical method could be used to substitute for the standard method provided that using the single seed specimen should be carefully considered.

Table 4 showed the calculated least sample sizes to keep the difference between sample mean and population mean at 0.010. 0.015, 0.020, 0.025, 0.030, 0.035, 0.041g dried tofu yield per gram seeds which was equivalent to the relative accuracy approximately 2.8%, 4.2%, 5.6%, 6.9%,

Table 2. The means and mean squares of fresh tofu yield of various treatments.

Size of specimen		1 seed	2 seeds	4 seeds	6 seeds	8 seeds	10 seeds	50g seeds
Tongzhou-Dou	$\bar{x}$	1.354	1.333	1.354	1.276	1.304	1.366	1.366
	s	0.048	0.164	0.270	0.273	0.152	0.246	0.171
	t	0.214	0.441	0.119	0.882	0.857	0.000	
	F	0.079	0.915	2.497	2.552	0.789	2.063	
	c.v.	3.55%	12.28%	19.97%	21.42%	11.65%	17.99%	12.52%
Nannong 88-48	$\bar{x}$	1.304	1.302	1.228	1.273	1.229	1.334	1.312
	s	0.041	0.122	0.176	0.177	0.130	0.182	0.103
	t	0.227	0.198	1.302	0.601	1.580	0.332	
	F	0.154	1.387	2.881	2.927	1.474	3.101	
	c.v.	3.11%	9.37%	14.32%	13.92%	10.58%	13.67%	7.90%
N21534	$\bar{x}$	1.684	1.704	1.690	1.537	1.651	1.673	1.596
	s	0.101	0.213	0.188	0.158	0.174	0.205	0.141
	t	1.609	1.578	1.346	0.884	0.778	0.981	
	F	0.514	2.287	1.794	1.256	1.529	2.118	
	c.v.	5.99%	12.48%	11.14%	10.25%	10.53%	12.23%	8.81%
Pooled Data	$\bar{\bar{x}}$	1.447	1.446	1.424	1.362	1.375	1.458	1.425
	s	0.184	0.248	0.287	0.238	0.238	0.257	0.185
	t	0.462	0.372	0.016	1.146	0.908	0.570	
	F	0.987	1.796	2.413*	1.650	1.657	1.935*	
	c.v.	12.70%	17.15%	20.18%	17.45%	17.32%	17.65%	12.98%

Table 3. The means and mean squares of dried tofu yield of various treatments.

Size of specimen		1 seed	2 seeds	4 seeds	6 seeds	8 seeds	10 seeds	50g seeds
Tongzhou -Dou	$\bar{x}$	0.364	0.350	0.375	0.360	0.369	0.356	0.371
	s	0.152	0.035	0.025	0.011	0.007	0.023	0.043
	t	0.140	1.200	0.255	0.786	0.146	0.980	
	F	12.53*	0.664	0.347	0.060	0.024	0.280	
	c.v.	41.73%	9.99%	6.74%	2.93%	1.78%	6.38%	11.56%
Nannong 88 - 48	$\bar{x}$	0.336	0.342	0.348	0.339	0.336	0.350	0.341
	s	0.045	0.019	0.018	0.031	0.021	0.015	0.022
	t	0.318	0.109	0.987	0.167	0.523	1.086	
	F	4.296*	0.801	0.727	2.098	0.964	0.477	
	c.v.	13.31%	5.65%	5.29%	9.22%	6.31%	4.26%	6.33%
N21534	$\bar{x}$	0.375	0.368	0.372	0.388	0.365	0.373	0.376
	s	0.025	0.035	0.019	0.023	0.027	0.020	0.022
	t	0.095	0.620	0.441	1.214	0.991	0.319	
	F	1.318	2.902	0.745	1.076	1.529	0.875	
	c.v.	6.65%	9.40%	5.04%	5.80%	7.50%	5.44%	5.77%
Pooled Data	$\bar{\bar{x}}$	0.360	0.353	0.365	0.362	0.357	0.360	0.363
	s	0.046	0.031	0.022	0.031	0.025	0.031	0.032
	t	0.294	1.226	0.283	0.124	0.818	0.369	
	F	2.106*	0.983	0.488	0.940	0.606	0.967	
	c.v.	12.79%	8.91%	6.07%	8.50%	6.91%	8.66%	8.74%

Table 4. The calculated least sample size of microanalytical method for various accuracy criteria of dried tofu yield under 95% confidence coefficient.

Accuracy criteria (g tofu/g seeds)	1 seed	2 seeds	4 seeds	6 seeds	8 seeds	10 seeds	50g seeds
0.010	89.2	41.6	20.7	39.8	25.6	40.9	42.4
0.015	39.6	18.5	9.2	17.7	11.4	18.2	18.8
0.020	22.3	10.4	5.2	10.0	6.4	10.2	10.6
0.025	14.3	6.7	3.3	6.4	4.1	6.5	6.8
0.030	9.9	4.6	2.3	4.4	2.8	4.5	4.7
0.035	7.3	3.4	1.7	3.3	2.1	3.3	3.5
0.040	5.6	2.6	1.3	2.5	1.6	2.6	2.6

8.3%, 9.7%, and 11.1% of 0.360g/g seeds, the assumed population mean based on the pooled data. It was obvious that the required sample sizes of microanalytical method of 2, 4, 6, 8, and 10 seeds were not larger than that of standard method to keep a same accuracy, but for the single seed method, double of the sample size was needed. For example, for standard method and 2, 4, 6, 8, 10 seeds methods, four replications are needed to keep the difference between sample mean and population mean less than 0. 035g dried tofu yield per gram seeds (about 10% relative accuracy), but for the single seed method, seven replications are required. Therefore, the microanalytical method with single seed could be used, but the sample size should be increased.

In microanalytical method, the analysis should be on a micro specimen or individual plant even individual seed basis, some steps of traditional preparation of tofu must be improved. The major point of innovation of the analytical method was to use centrifuge to replace filtering and compressing of the coagulated material in soymilk. The microanalytical method can be applied to study the variation of tofu yield among individual plants in plant generation. Further, the genetic variation of tofu yield among single seeds in seed generation can be also studied with this method in case that the sample size should be increased.

References

Lim, B. T., et al. 1990. Yield and quality of tofu as affected by soybean and soymilk characteristics, calcium sulfate coagulant. J. Food Sci. 55(4): 1088 -1092.

Wang, H. L. 1983. Effect of soybean varieties on the yield and quality of tofu. Cereal Chem. 60(3): 245-248.

Zhou, X. A., J. Y. Gai, and Y. H. Ma. 192. Variation of tofu processing traits and their relationship with components of seed storage protein in soybean. Soybean Science. 11(4): 283 - 289.

Zhong Gao

Junyi Gai

JILIN ACADEMY OF AGRICULTURAL SCIENCES
Gongzhuling
Jilin province
The People's Republic of China

A Study on Resistance to Soybean Mosaic Virus and *Aphis Glycinece* of Perennial Wild Soybean

Introduction

Soybean mosaic virus (SMV) and *Aphis glycinece* are the major disease and insect in soybean planting area of China. Therefore, evaluation of special genotypes of resistance to SMV and *Aphis glycinece* is an important object in soybean breeding. Both perennial wild soybean in subgenus glycine and annual soybean in subgenus soja are belonged to genus Glycine. The screening on germplasms of resistance to SMV and *Aphis glycinece* has been carried out by some institute in our country. Yaoderong et al. screened three germplasms, which have higher resistance to SMV than cultivated soybean. From Chinese wild soybean; Sunyongji et al. screened two germplasms of resistance to SMV, five germplasms which it's seed is virus-free from wild soybean, this enriched the gene resources of soybean breeding.

Genus Glycine includes subgenus glycine and soja. The application of perennial wild soybean in soybean breeding has been explored by several researchers, and good progresses have been made. Accompanying the rapid development of gene manipulation, the application of perennial wild soybean in soybean breeding will come to be true. Our purpose of this study is to identify the resistance of perennial wild soybean to disease and insect, and to provide new gene resources for soybean breeding.

Materials and Methods

Germplasms abroad were kindly provided by Dr. T. Hymowitz in U.S.A. and Dr. A. H. D. Brown in Australia. The germplasms of China were provided by professor Lifushan.

I. Identification of resistance to SMV

The perennial wild soybeans were sowed in flowerpot on July 9th, 1991. Five of the flowerpots for each genotype and 2-3 plants per pot. Leaves were inoculated with mixed SMV strains at seedling stage, symptoms were investigated and resistance were evaluated on August 31st refereed to the standard of seven-class classification. It was identified again in 1992 and some new species and two annual wild soybeans were added. Since most of the germplasms showed higher resistance to SMV, we inoculated strain No.1 and strain No. 3

separately, others were the same as that in 1991. The classification standard of resistance to SMV are as follows:

0: high resistant, no symptoms

1: resistant, plant develops and pods normally

2: middle resistant, plant develops and pods near normally

3: middle susceptible, wrinkled spotted spotted leaves, plant is not or slightly shortened, the number of podding decreased lightly, a few of the blooms are short and less

4: susceptible, seriously wrinkled

5: high susceptible, dwarfed plant

6: extremely susceptible, top necrosis

II. Identification of resistance to *Aphis glycinece*

The experimental accessions were sowed on May 31th, 1991, and the planting method was the same as that for SMV. Five flowerpot for each genotype and five plants per pot. They were transferred to hot-house, which was 20 meters long, 5.4 meters wide and 1.8 meters high. The net had 40 meshes per cubic centimeter. The aphis on plants were removed before they were transferred. The aphis were inoculated on July 19th. The degree of injured and plant number with aphis were investigated and then the injury index was calculated. The classification of injury degree and the calculation of injury index are as follows:

Classification of injury degree:

0: plant develops normally, there is no aphis

1: plant develops normally, there are a few aphis

2: plant develops near normally, there are more aphis on the younger stem and leaves on the top

3: Leaves are slightly wrinkled, there are aphis anywhere on the younger stem and leaves

4: plant is shortened, leaves turn yellow and are seriously wrinkled.

$$\text{Injury index} = \frac{\sum (\text{injury degree class} \times \text{relative number of plant})}{4 \times \text{total number investigated}}$$

This was repeated in 1992 and some new species were added.

At last based on the range of injury index of all samples, the resistance to aphis were divided into five classes, they are: high resistant (HR), resistant (R), meadium type (M), susceptible (S), and high susceptible (HS).

I. Resistance to SMV

Twelve accessions in eight species were identified in 1991, most of them showed high resistance to SMV except two accessions in *G. tabacina*. The results inoculated by strain showed that nine accessions immune to SMV. To sum up the results of two years, three accessions are highly resistant to SMV, they are *G. canescens*, *G. clandestina* and *G. tomentella* (2N=80).

Table 1. Identification of resistance of perennial wild soybean to SMV

Species	1991 Mixed Strains	1992 Strain No. 1.	1992 Strain No. 3
G. argyrea	0	1	1
G. canescens	0	0	0
G. clandestina	0	0	0
G. curvata	-	0	0
G. cyrtoloba	0	1	1
G. falcata	-	1	Necrosis
G. latifolia	0	3	2
G. microphylla	0	0	1
G. tabacina (2N=40)	1	1	1
G. tabacina (2N=80)	0	1	3
G. tabacina (China)	4	2	3
G. tomentella (2N=40)	0	1	1
G. tomentella (2N=78)	-	1	1
G. tomentella (2N=80)	0	0	0
G. tomentella (China)	0	1	1
G. soja No. 1	-	4	4
G. soja No. 2	-	4	4

II. Resistance to *Aphis glycinece*

The result in table 2. showed that: 12 accessions in nine species were identified in 1991, except for G. tabacina (2N=80) was resistant to aphis, others were all highly resistant. Nine accessions were highly resistant in 1992. Seven germplasms were highly resistant during two years, they are *G. clandestina, G. cyrtoloba, G. falcata, G. latifolia, G. microphylla, G. tomentella* (2N=40), and *G. tomentella* (2N=78).

We had identified the resistance to SMV of 2341 cultivated soybean germplasms from northeast of China and screened one resistant accession and many middle resistant accessions. Two resistant and 11 middle resistant accessions were screened from more than 800 wild soybean germplasms. But no genotype was found which is highly resistant to SMV. Now we found three accessions of high resistance to SMV from 15 perennial wild soybean germplasms, this proves that perennial wild soybean germplasms have higher resistance to SMV and we should pay more attention to them.

There is no cultivated germplasm of high resistance to aphis except few bare soybeans, Yaoderong et al. had screened three genotypes of high resistance from wild soybean germplasms in China. Seven perennial wild soybean germplasms have been found from only 15 samples, and their injury index is lower than that of annual wild

Table 2. Identification of resistance of perennial wild soybean to *Aphis glycinece*

Species	1991		1992	
	Injury index	Resistant level	Injury index	Resistant level
G. argyrea	25.0	HR*	55.0	R
G. canescens	14.2	HR	100.0	HS
G. clandestina	25.0	HR	25.0	HR
G. curvata	-	-	50.0	HR
G. cyrtoloba	1.0	HR	25.0	HR
G. falcata	10.	HR	25.0	HR
G. latifolia	34.4	HR	50.0	HR
G. microphylla	16.7	HR	25.0	HR
G. tabacina (2N=40)	6.9	HR	68.8	M
G. tabacina (2N=80)	50.9	R	56.3	R
G. tabacina (China)	-	-	79.7	S
G. tomentella (2N=40)	25.0	HR	25.0	HR
G. tomentella (2N=78)	25.0	HR	50.0	HR
G. tomentella (2N=80)	-	-	25.0	HR
G. tomentella (China)	25.0	HR	70.3	M

soybean of high resistance, this proved that perennial wild soybean germplasms also have higher resistance to *Aphis glycinece.*

There are highly resistant accessions in perennial wild soybean germplasms, it should be pay attention to in soybean breeding, especially in molecular breeding and biological engineering.

References

Wangchenglun et al. 1962. Study on *Aphis glycinece*. Chinese Journal of Entomology 11(1) : 31-44.

Sunyongji et al. 1991 Study on the resistance of wild soybean to soybean mosaic virus Soybean Science 10(3): 212-216.

Liuzhonglin et al. 1984 Evaluation method on the resistance of soybean to soybean mosaic virus. Jilin Agricultural Science (1) : 44-46.

Hujicheng et al. 1984 Preliminary identification of soybean mosaic virus of wild soybean. Chinese Journal of Plant Pathology. 14(2): 122-123.

Yaoderong et al. 1988. Study on the method of identification of resistance to *Aphis glycinece* of wild soybean. Jilin Agricultural Science (3): 1-3.

Yaoderong et al. 1989. Screening on the resistance of wild soybean to *Aphis glycinece*. Jilin Agricultural Science (3): 15-19.

Zhuang Bingchang
Sun Yongji
Lu Qinhua
Wang Yumin
Xu Bao

FUDAN UNIVERSITY
Biochemistry Department
Shanghai, China, 200433

JILIN ACADEMY OF AGRICULTURAL SCIENCE
Jilin, China 136100

GANSU ACADEMY. OF AGRICULTURAL SCIENCE
Gansu, China 730070

Biochemical Analyses of a New Electrophoretic Variant of SBTi-A_2 from Soybean (*G.max*) Seed Storage Protein

Introduction

The Kunitz trypsin inhibitor was isolated and crystallized by Kunitz in 1945 from soybean seed protein. It was designated SBTi-A_2 through electrophoreses and genetics study. There were three alleles, Ti^a, Ti^b, Ti^c, in this locus. Recently, Zhao Shuwen (1992) reported that a new variant of SBTi-A_2, Ti^x, was found from soybean seed in Gansu province of China. The Ti^x protein was purified and some biochemical analyses have been done in our laboratory.

Materials and methods

Ti^a and Ti^x were extracted from corresponding soybean seeds respectively, following the method modified by Mihoko Yamamoto (1967), and purified by DEAE-52 column (0--0.4M NaCl, 50mM TrisCl, pH8.0). The molecular weight of inhibitors was determined by SDS-PAGE and N-termination residue was identified by using DNS-CL. The trypsin inhibitory activity of the two inhibitors was analyzed by BAEE method (Seung-Ho KIM, 1992) After repurified by FLPC and the amino acid composition of the polypeptides was performented by amino acid analyzer.

Results

The quality of purified Ti^a and Ti^x was suitable for biochemical analyses. The results of SDS-PAGE and DNS-Cl analysis showed that Ti^x is a 21 kDa polypeptide (Fig. 1) with Asp as N-terminal residue. These are the same as those of Ti^a . The titration curves showed that Ti^x and Ti^a also inhibited porcine trypsin at a ratio of 1:1, but the K_1 value of Ti^x was a little higher than that of Ti^a (Fig. 2). The amino acid compositions indicated that there is a difference of two or three residues between Ti^x and Ti^a (Table 1)

Discussion

According to our study, Ti^x has the same MW, N-terminal residue and profile of titration curve as Ti^a , but there are different amino acid composition and trypsin inhibition property between Ti^x and Ti^a . In comparison with Ti^a , Ti^x has one more Lys,

one more Arg, one less Glu (or Gln), and maybe one less Asp (or Asn). Then Ti^x has one more Lys, one more His compared with Ti^b (Seung-Ho KIM, 1985) The changes of amino acid composition make Ti^x possess less negative charge and migrate more slowly than Ti^a and Ti^b in PAGE. It was consistent with the pattern of inhibitors on polyacrylamide gel. The results supported the conclusion that Ti^x is a fourth allele of SBTi-A_2 from soybean seed storage proteins (Zhao Shuwen, 1992)

---

* Author to whom correspondence should be addressed.

References

Mihoko Yamamoto and Tokuji Ikenaka. 1967. The Journal of Biochem. 67(2): 141-149

Seung-Ho KIM et al., 1985. J. Biochem. 98(2) : 435-448

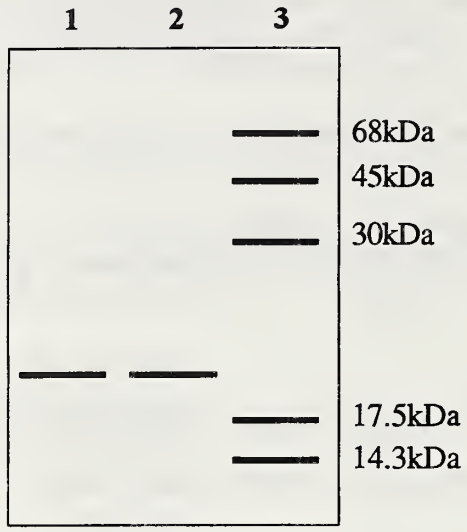
Zhao Shuwen, and Wang Hai. 1992. Soybean Genetics Newsletter. 19: 22-24.

Yan Qingyan
Gao Kaiming
Gu Qimin
Zhao Shuwen
Wang Hai

Table 1. Amino acid composition of Ti^x and Ti^a from soybean seeds. The number of residues in parenthesis is from the established sequences.

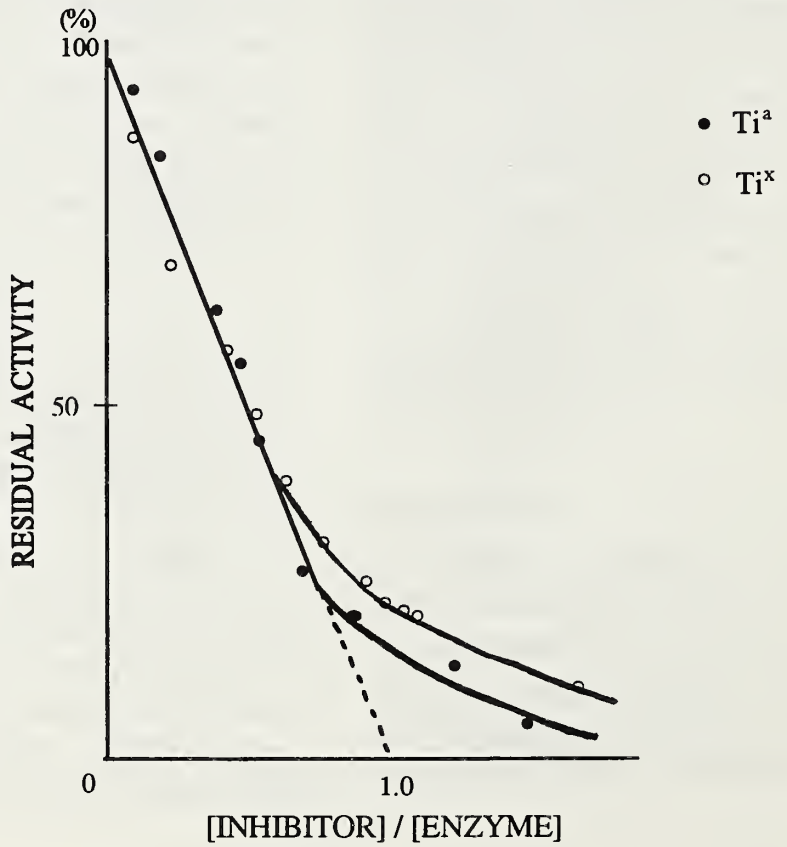
Amino Acid	Ti^a	Ti^x	Ti^x - Ti^a
Asp	26.4 (26)	25.9	-0.5
Thr	6.2 (7)	6.0	
Ser	8.9 (11)	8.4	-0.5
Glu	19.0 (18)	18.0	-1.0
Pro	10.6 (10)	10.7	
Gly	16.7 (16)	17.0	
Ala	8.9 (8)	9.3	
Val	13.9 (14)	13.8	
Mel	1.7 (2)	1.9	
Ile	14.0 (14)	13.6	
Leu	14.8 (15)	14.5	
Tyr	3.9 (4)	4.3	
Phe	9.1 (9)	8.5	-0.6
His	1.8 (2)	1.8	
Lys	10.3 (10)	11.1	+0.8
Arg	8.7 (9)	10.0	+1.3

Figure1. 15% SDS-PAGE analysis of Ti^x and Ti^a.



1. Ti^a
2. Ti^x
3. MW markers

Figure 2. Inhibitory activities of trypsin inhibitors, Ti^a,Ti^x. A fixed amount of porcine trypsin was mixed with increasing amounts of the inhibitors.



JILIN ACADEMY OF AGRICULTURAL SCIENCE
Gongzhuling of Jilin Province, China 136100

GANSU ACADEMY OF AGRICULTURAL SCIENCE
Gansu, China 730070

FUDAN UNIVERSITY
Biochemistry Department
Shanghai, China 200433

Inheritance of a new SBTi--A_2 variant in seed protein of soybean (*G. max)*

A new variant of SBTi--A_2, Ti^d, originally designated as Ti^x (1995), was discovered in 1992(1992), and it was crossed with lines having Ti^a, Ti^b, Ti^c, and titi, respectively. The hereditary of electro-phoretic band of the new variant in F_2, F_3, and F_4 seeds was studied.

Materials and Methods

F_2 seeds from Ti^a x Ti^b, Ti^d x Ti^a; Ti^b x Ti^d, Ti^d x Ti^b; Ti^c x Ti^d, Ti^d x Ti^c; and titi x Ti^d, Ti^d x titi; were analyzed by polyacrylamide gel electrophoresis.

F_3 seeds derived from the F_2 seeds of Ti^b x Ti^d, with Ti^b and Ti^d bands, and F_4 seeds from the F_3 seeds, with Ti^b and Ti^d, were further analyzed for electrophoretic band. A slice of seed cutting from each sample of seeds away from its embryo was used in the electrophoresis.

Result and Discussion

Ti^d was crossed with other alleles of SBTi--A_2(Ti^a, Ti^b, Ti^c, and titi). The zymogram patterns of reciprocal crosses were the same. The detection by adding trypsin and Rf value confirmed that these bands were the expression of parents gene (1995. a.b.)

1. Reciprocal crosses of Ti^d with Ti^a, Ti^b, Ti^c: The segregation of F_2 seeds were fitted to the ratio of 1:2:1.

Table 1. Observed and expected segregation of electrophoretic bands of SBTi--A_2 in F_2 seeds from selfed F_1 soybean plants from the reciprocal crosses between Ti^a x Ti^d.

Crosses	No. F_2 seeds		Electrophoretic bands of F_2 seeds			X^2	Probability
		Rf	0.85	0.85/0.79	0.79		
			Ti^a	Ti^a/ Ti^d	Ti^d		
Ti^a x Ti^d	268	Observed	60	154	54	6.238	≈ 0.04
	198		45	105	48	2.954	≈ 0.50
	268	Expected	67	134	67		
	198		49.5	90.0	49.5		
		Rf	0.79	0.79/0.85	0.85		
			Ti^d	Ti^d/Ti^a	Ti^a		
Ti^d x Ti^a	166	Observed	31	97	38	3.124	> 0.10
		Expected	41.5	93.0	41.5		

Table 2. Observed and expected segregation of electrophoretic bands of SBTi--A_2 in F_2 seeds from selfed F_1 soybean plants from the reciprocal crosses between Ti^b x Ti^d

Crosses	No. of seeds		Electrophoretic bands of F_2 seeds			X^2	Probability
		Rf	0.82 Ti^b	0.82/0.79 Ti^b/ Ti^d	0.79 Ti^d		
Ti^b x Ti^d	212	Observed	51	97	64	3.122	≈ 0.25
		Expected	53	106	53		
		Rf	0.79 Ti^b	0.79/0.85 Ti^b/Ti^d	0.85 Ti^d		
Ti^d x Ti^b	135	Observed	37	71	27	1.844	≈0.50
		Expected	33.75	67.50	33.75		

Table 3. Observed and expected segregation of electrophoretic bands of SBTi--A_2 in F_2 seeds from selfed F_1 soybean plants from the reciprocal crosses between Ti^c x Ti^d

Crosses	No. of F_2 seeds		Electrophoretic bands of F_2 seeds			X^2	Probability
		Rf	0.87 Ti^c	0.87/0.79 Ti^c/ Ti^d	0.79 Ti^d		
Ti^c x Ti^d	167	Observed	41	81	45	0.341	>0.50
		Expected	41.75	83.50	41.75		
		Rf	0.79 Ti^d	0.79/0.85 Ti^d/Ti^c	0.85 Ti^c		
Ti^d x Ti^c	107	Observed	29	56	22	1.149	<0.50
		Expected	26.75	5305	26.75		

2. Reciprocal cross of Ti^d with titi: The segregation of F_2 seeds were fitted to the ratio of 3 : 1.

Table 4. Observed and expected segregation of electrophoretic bands of SBTi--A_2 in F_2 seeds from selfed F_1 soybean plants from the reciprocal crosses between titi x Ti^d

Crosses	No. of F_2 Seeds		Electrophoretic bands of F_2 seeds		X^2	Probability
		Rf		Ti^d=0.79		
			titi	Ti^d		
titi x Ti^d	444	Observed	115	329	0.192	>0.90
		Expected	111	333		
		Rf		Ti^d=0.79		
			Ti^d	titi		
Ti^d x titi	284	Observed	218	66	0.469	>0.50
		Expected	213	71		

3. The F_2 seeds of Ti^b x Ti^d which had both Ti^b and Ti^d bands were planted and F_3 seeds were collected for electrophoretic analysis, showing segregating ratio 1 : 2 : 1. We sowed F_2 seeds only having male or female parent band, the gathered F_3 seeds also had one type of male or female parent.

Table 5. Observed and expected segregation of F_3 seeds from selfed F_2 soybean plants of Rf0.82, Rf0.82/Rf0.79, and Rf0.79 electrophoretic band of SBTi--A_2 from the cross Ti^b x Ti^d.

No. of F_3 seeds		F_3 electrophoretic Rf0.82 Ti^b	Rf0.82/Rf0.79 Ti^b/Ti^b	Rf0.79 Ti^d	X^2	Probability
F_3 Type Rf0.82/Rf0.79						
194	observed	5.3	95	46	0.588	>0.50
	expected	48.5	97	48.5		
F_3 Type Rf0.82						
133	observed	133	0	0		
	expected	133	0	0		
F_3 Type Rf0.79						
139	observed	0	0	139		
	expected	0	0	139		

4. F_3 seeds of Ti^b x Ti^d which had Ti^b and Ti^d bands were sowed for the propagation of F_4 seeds, and the segregation of electrophoretic bands also coincided to 1: 2: 1. F_3 seeds, which only had Ti^b or Ti^d, were sowed. The F_4 seeds also had Ti^b or Ti^d.

Table 6. Observed and expected segregation of F_4 seeds from selfed F_3 soybean plants of Rf0.82, Rf0.82/Rf0.79 and Rf0.79 electrophoretic bands of SBTi--A_2 from the cross Ti^b x Ti^d.

No. of		F_4 electrophoretic			X^2	Probability
F^4 Seeds		Rf0.82 Ti^b	Rf0.82/Rf0.79 Ti^b/Ti^d	Rf0.79 Ti^d		
F_4 Type Rf0.82/Rf0.79						
104	observed	27	49	28	0.365	<0.90
	expected	26	52	26		
F_4 Type Rf0.82						
142	observed	142	0	0		
	expected	142	0	0		
F_4 Type Rf0.79						
78	observed	0	0	78		
	expected	0	0	78		

We can reach the following conclusion from our study:

1. The difference of reciprocal crosses does not exist in zymogram pattern of F_1 seeds, showing that the new variant of SBTi--A_2 (Ti^d) is controlled by the nuclear gene.

2. The result of electrophoresis of F_1 and F_2 seeds showed that the new variant was dominant to SBTi--A_2 null and was co-dominant to Ti^a, Ti^b, and Ti^c types.

3. Further data of electrophoresis in F_3 and F_4 seeds proved that the new variant of SBTi--A_2 had a stable heritability.

We concluded that the new variant of SBTi--A_2 was the fourth of SBTi--A_2 alleles and designated as Ti^d, based on its genetical and biochemical studies.

References

Zhao Shuwen and Wang hai. 1992. Soybean Genetics Newsletter. Vol. 19: 22-29.

Zhao Shuwen et al. 1995a. Soybean Genetics Newsletter. Vol. 22: 83-84.

Zhao Shuwen et al. 1995b. Soybean Genetics Newsletter. Vol. 22: 85-88.

Yan Qingyan et al. Biochemical analyes of a new electrophoreticvariant of SbTi--A_2 from soybean (*G. max*) seed storangorage. protein. (in press)

Zhoa Shuwen
Meng Xiangxun
Gu Qimin
Gao Kaiming
Wang Hai
Wang Hao

NANJING AGRICULTURAL UNIVERSITY
Soybean Research Institute
Nanjing, Jiangsu 210095
The People's Republic of China

Inheritance of Short Petiole in Soybeans

Introduction

Short petiole trait was thought to be useful to improve soybean yield by increasing density. Kilen (1983) reported short petiole of D76-1609 was controlled by a recessive gene. Tian (1988) found another short petiole line with a single recessive gene. A new mutant with short petiole NJ90L-1SP was found in Nanjing Agricultural University in 1993, and its short petiole was controlled by two duplicate recessive genes (You et al. 1995). The objective of this study was to reveal the inheritance of short petiole of D76-1609 crossed with cultivars from Jiangsu province of China and genetic relationship between the two short petiole parents, D76-1609 and NJ90L-1SP.

Materials and Methods

Six crosses and their parents were shown in Table 1. The short petiole parents were D76-1609 and NJ90L-1SP and the long petiole parents were Nannong 88 - 48, Hongyin 1, NJ90L-2 and NJ87-23 from Jiangsu province. The crosses were made in 1993. Part of hybrid seeds were sent to Hainan island to get F_2 and F_3 during the winter of 1993 and spring of 1994. The P_1, P_2, F_2 (including reciprocal F_1), F_2 and F_3 lines were evaluated at Jiangpu Agricultural Experimental Station of Nanjing Agricultural University in 1994 and 1995 with a row length of 1.8m and row spacing of 0.5m. The length of the top third leaf petiole of cross 1, 3, 6 (determinate X determinate) and the longest leaf petiole on main stem of other three crosses(indeterminate or semi-determinate X determinate) were measured in 1994. At the very beginning the single petiole length was thought to be used to classify the segregation types. But unfortunately it was difficult to use that kind of criterion due to that the petiole length varied from leaf to leaf and from stem termination type to stem termination type. However, it was easy to classify the segregating types of long and short petiole by observing the whole plant leaves. The plant was classified into short petiole type if all the leaves from bottom to top of a plant looked short, and into long if all looked long. In this way, it was easy to observe and make a good classification.

Results and Discussion

1. Inheritance of short petiole of D76-1609:

Cross 1 through cross 5 were made with a short petiole parent, D76-1609. The petiole length of F_1 of the five crosses was similar to each of their long petiole parents which

indicated that the long petiole was dominant to short petiole.

The segregation of petiole types in F_2 of cross 1 and cross 2 showed a good fit to 3 long: 1 short in 1994 and 1995 (Table 2.) The proportion of long petiole F_3 lines plus segregating lines to short petiole lines fitted a 3:1 ratio in 1994. In 1995, among 96 F_3 lines of cross 1, there were 26 lines with short petiole which could be traced back to short petiole F_2 plants, and there were 23 lines with long petiole and 47 segregating lines which fitted a 1:2 ratio (x^2 = 0.01, P>0.9). In cross 2, among 67 F_3 lines, there were 16 lines with short petiole corresponding to short petiole F_2 plants and there were 20 lines with long petiole and 31 segregating lines which fitted the 1:2 ration (x^2 = 0.55, P = 0.25 - 0.50). The results of the two crosses indicated there was one pair of gene difference between the long and short petiole parents.

In other three crosses with D76-1609, the segregation of petiole types fitted a ratio of 15 long : 1 short in F_2 in 1994 and in 1995 (Table 2.). The proportion of long petiole and segregating F_3 lines to short petiole lines also fitted 15:1 in 1994. In 1995, among 111 F_3 lines of cross 3, there were 4 lines with short petiole corresponding to their short petiole F_2 plants, and there were 47 lines with long petiole and 60 segregating lines which fitted a ratio 7:8 (x^2 = 0.22, P = 0.50-0.75). In cross 5, among 60 F_3 lines, there were 8 lines with short petiole corresponding to short petiole F_2 plants and there were 20 lines with long petiole and 32 segregating lines which also fitted the ratio of 7:8 (x^2 = 1.1, P = 0.25-0.50). The results of the three crosses indicated a two duplicate genes inheritance and there was a second gene controlling short petiole.

2. Results from NJ90L-1SP X D76-1609, the cross between two short petiole parents.

The F_1 and reciprocal F_1 of the cross were long petiole, indicating that the genes controlling the short petiole were not allelic in the two parents, and there probably existed gene interaction. The segregation of long petiole to short petiole in F_2 was 584: 75 and 725: 104 in 1994 and in 1995, respectively (Table 3.) Because short petiole of D76-1609 and NJ90L-1SP were controlled by one recessive gene or two recessive genes according to previous studies, there must be more than two loci involved. If there were three loci determining the trait, the expected ratio of long to short petiole must be 9:7, and if four loci were involved, it should be 225:31. In the experiment, the segregation of long petiole to short petiole in F_2 was 584:75 and 725:104 in 1994 and in 1995, respectively (Table 3.) which did fit the ratio of 224:31, but not 9:7. Theoretically the 31 short petiole from 256 individuals in F_2 should include 15 with characteristics like D76-1609, 15 with short petiole and abnormal pulvinus characteristics of NJ90L-1SP and 1 recombination type which could not be distinguished. So the proportion of short petiole plants like D76-1609 to those like NJ90L-1SP should be about 1:1. In fact, the real proportion was 42:33 in 1994 (x^2 = 0.86, P = 0.25-0.50) and 46:58 in 1995 (x^2 = 1.06, P = 0.25-0.50) which demonstrated that there were at least four genes controlling the length of petiole between D76-1609 and NJ90L-1SP. Short petiole of each parent was controlled by two duplicate recessive

genes that were not allelic. There existed complementary interaction between each two of the four loci.

References

Egli, D. B. 1988. Plant density and soybean yield. Crop Sci. 28: 977-981.

Kilen, T. C. 1983. Inheritance of a short petiole trait in soybean. Crop Sci. 23: 1208-1209.

Peizhan, T., W. Jian and S. Zhiqiang. 1988. Inheritance of short petiole character in soybean. Soybean Sci. (in Chinese) 7(4): 341-343.

You M., T. Zhao, and J. Gai. 1995. Performance and inheritance of short petiole trait of a new soybean mutant. SGN 22: 71-75.

Junyi Gai

Mingan You

Tuanjie Zhao

Table 1. Crosses and their parents tested

Code of cross	Female			Male		
	Name	petiole	stem termination	Name	petiole	stem termination
1	Nannony86-4	long	det.	D76-1609	short	det.
2	Hongyin 1	long	indet.	D76-1609	short	det.
3	NJ90L-2	long	det.	D76-1609	short	det.
4	NJ87-23	long	indet.	D76-1609	short	det.
5	Nannong 88-48	long	semi-det.	D76-1609	short	det.
6	NJ90L-1SP	short	det.	D76-1609	short	det.

Table 2. Segregation of petiole in crosses with D76-1609

	Cross	Year	Generation	No. of total plants (lines)	No. of plants (lines) with LP	No. of Plants (lines) with SP	Expected ratio	x^2	P
1	Nannong86-4	1994	F_2	607	470	137	3 : 1	1.79	0.10~0.25
	X		F_3	152	121*	31	3 : 1	1.49	0.10~0.25
	D76-1609	1995	F_3	96	70*	26	3 : 1	0.12	0.50~0.75
2	Hongyin No. 1	1994	F_2	250	197	53	3 : 1	2.59	0.10~0.25
	X		F_3	203	148*	55	3 : 1	0.37	0.50~0.75
	D76-1609	1995	F_3	67	51*	16	3 : 1	2.34	0.10~0.25
3	NJ90L-2	1994	F_2	407	373	34	15 : 1	2.73	0.05~0.10
	X		F_3	114	109*	5	15 : 1	0.39	0.50~0.75
	D76-1609	1995	F_2	537	507	30	15 : 1	0.20	0.50~0.75
			F_3	111	107*	4	15 : 1	1.11	0.25~0.50
4	NJ87-23	1994	F_2	237	215	22	15 : 1	3.23	0.05~0.10
	X		F_3	149	135*	14	15 : 1	1.92	0.10~0.25
	D76-1609	1995	F_3	60	52*	8	15 : 1	4.07	<0.05
5	Nannong88-48	1994	F_2	269	247	22	15 : 1	1.40	0.10~0.25
	X		F_3	62	58*	4	15 : 1	0.04	0.75~0.90
	D76-1609	1995	F_2	132	119	13	15 : 1	2.34	0.10~0.25
			F_3	65	61*	4	15 : 1	0.10	0.75~0.90

Note: LP - long petiole, SP - short petiole. The same is true for the later table.
* : including long petiole lines and segregating lines

Table 3. Segregation of the cross between two short petiole parents NJ90L-1SP and D76-1609 (1994)

Generation	No. of total plants	No. of plants with LP	No. of plants with SP	Expected ratio	x^2	P
P_1	18		18			
P_2	27		27			
F_1	5	5				
F_{1R}#	5	5				
F_2	659	584	75	225 : 31	0.26	0.75~0.90
F_2##	829	725	104	225 : 31	0.05	0.75~0.90

F_{1R} represents reciprocal F_1.
The data observed in 1995.

NANJING AGRICULTURAL UNIVERSITY
Soybean Research Institute
Nanjing, Jiangsu 210095
The People's Republic of China

Inheritance of the Zigzag Stem and Dwarf Stature of PI227224 Performed in Nanjing

Introduction

The brachytic stem of PI227224 was first reported by Kilen and Hartwig (1975), and was thought to be useful to reduce lodging and create new plant-type. Kilen (1977) found a pair of recessive gene controlling the trait. The brachytic stem performed as shortened internodes with zigzag arrangement of main stem. Later, Boerma and Jones (1978) identified a second gene. Umezaki (1991) reported the dwarf stature was controlled by a recessive gene. Some evaluations did not show great advantage of transferring it to several high productive genetic background. The present study was to determine the inheritance of zigzag stem and dwarf stature of PI227224 performed in Nanjing.

Materials and Methods

PI227224 was crossed to each of two tall and normal-stem genotypes: NJ863070, a determinate line from Huanghuai area of China (cross 1), and D76-1609, a determinate line with short petiole from Mississippi of USA (cross 2) in 1993. Part of hybrid seeds were sent to Hainan island to get F_2 and F_3 seed during the winter of 1993 and spring of 1994. Seeds of P_1, P_2, F_1, reciprocal F_1, F_2, reciprocal F_2 and F_3 lines were planted at Jiangpu Agricultural Experimental Station of Nanjing Agricultural University of June 15, 1994 with row length 1.8m and row spacing 0.5m. Plants of each generation were harvested and the stem types (normal-stem or zigzag-stem), and plant height were investigated.

Results and Discussion

Results showed that the zigzag-stem was recessive to normal-stem. The segregation of the two stem types in F_2 indicated a good fit to three normal-stem : one zigzag-stem (Table 1). In NJ863070 X PI227224, the proportion of the two stem types was 124 normal : 39 zigzag in F_2 which was confirmed by the F_3 data that the proportion of F_3 lines with normal stem and segregating stem (normal plus zigzag) to lines with zigzag stem was 108 : 30, fitting a 3 : 1 ratio ($x^2 = 0.61$, $P = 0.25\text{-}0.50$). Unfortunately, the ratio of normal lines : segregating lines was failed to fit a 1 : 2 ratio due to not enough number of plants in a line which caused difficulty to distinguish the normal lines from segregating lines. In D76-1609 X PI227224, the proportion of the two stem types was 336 normal : 132 zigzag in F_2, and 37 normal lines : 62 segregating lines : 25

zigzag lines in F_3 fitting a ratio of 3 : 1 and 1 : 2 respectively. The results indicated that a recessive gene controlling the zigzag-stem trait.

Table 2 listed the distribution of plant height in P_1, P_2, F_1, F_2, of two crosses. Plants with zigzag stem could cause reducing plant height, but the zigzag degree was low for the short internode, and the height was about 90% of the sum of all internodes on stem. When tall parents (P1) were crossed with dwarf line PI227224, the height of F1 was almost as tall as P1 indicating that tall was completely dominant to dwarf. The distribution of overlapped two peaks in F_2 of the two crosses showed that there existed a major gene and probably some minor genes controlling the trait. By using the valley value of 50cm to classify tall and dwarf types, the segregating proportion were 123 (tall) : 40 (dwarf) in cross 2 (Table 3.) which fitted a ratio of 3 : 1, and indicated a recessive gene for dwarf of PI227224.

All kinds of the recombination types of stem type and plant height were observed, but the ratio of tall with normal stem, tall with zigzag stem, dwarf with normal stem, and dwarf with zigzag stem in F_2 were 117: 6 : 7 : 33 in cross 1. and 435 : 32 : 25 : 139 in cross 2. significantly deviating from 9 : 3 : 3 : 1 (Table 4.). The recombination rate was 8.40 $\pm$ 2.3% in cross 1, and 9.53 $\pm$ 1.44% in cross 2 by using the maximum likelihood method, with a pooled value of 9.26 $\pm$ 1.58%.

References

Boerma H. R. et al. 1978 Inheritance of a second gene for brachytic stem in soybean. Crop Sci. 18(4): 559-560

Kilen T. C. and E. E. Hartwig 1975. A short internode character in soybean and its inheritance. Crop Sci. 15: 878-879.

Kilen. T. C. 1977. Inheritance of the brachytic character in soybeans. Crop Sci. 17: 853-854.

Kilen T. C. 1990. Brachytic stem and narrow leaflet effects on soybean seed composition and yield. Crop Sci. 30(5): 1006-1008.

Raudy W. et al. 1993. Soybean growth and light interception: response to differing leaf and stem morphology. Crop Sci. 33(3): 520-524.

Umezaki T. 1991. Inheritance and growth habits of two soybean dwarf lines Soybean Genetics Newsletter. 18: 154-158.

Walker D. B. et al. 1978. Morphological study of the mechanism causing brachytic stem in soybean. Can. J. Plant Sci. 58: 993-998.

Junyi Gai

Tuanjie Zhao

Table 1. Segregation of stem types in two crosses with PI227224

Cross	Generation	No. of total plants (lines)	No. of normal plants (lines)	No. of zigzag plants (lines)	Expected ratio	x^2	P
NJ863070	F_2	163	124	39	3:1	0.05	0.75~0.90
X	F_{2R}#	176	140	36	3:1	1.71	0.10~0.25
PI227224	F_3 lines	138	108##	30	3:1	0.61	0.25~0.50
D76-1609	F_2	468	336	132	3:1	2.39	0.10~0.25
X	F_{2R}	520	387	133	3:1	0.07	0.75~0.90
PI227224	F_3 lines	124	37nor.+62segr.	25zigzag	1:2:1	2.32	0.25~0.50

Note: # F_{2R} represents F2 from reciprocal F1.
including normal stem lines and segregating lines.

Table 2. Distribution of plant height in two crosses with PI227224 (cm)

Cross	Generation	20	20~25	25~30	30~35	35~40	40~45	45~50	50~55	55~60	60~65	65~70	70~75	75~80	80~85	85~90	90~95	95~100	N	$\bar{X}$	S
NJ863070	P_1									1		5							6	66.7	3.6
X	P_2		2	16															18	27.4	1.6
PI227224	F_1									3	1	1		1					6	62.3	7.7
	F_2		1	2	8	7	13	9	15	16	29	27	17	8	7	4			163	60.1	13.9
D76-1609	P_1									1	5	1							7	62.7	2.6
X	P_2		2	16															18	27.4	1.6
PI227224	F_1							2	2	6	8	5	3		1				27	62.6	8.0
	F_{1R}							1	2	7	6	6	1						23	62.0	5.8
	F_2	4	12	25	26	22	22	13	40	61	59	56	65	42	12	5	4		468	58.4	17.0

Table 3. Segregation of plant height in F2 of two crosses with PI227224

Cross	No. of total plants	No. of normal plants	No. of dwarf plants	Expected ratio	x^2	P
NJ863070 X PI227224	163	123	40	3:1	0.002	> .90
D76-1609 X PI227224	468	344	124	3:1	0.48	0.50~0.75

Table 4. Tests for linkageship between dwarf stature and zigzag stem in F_2

Cross	No. of four phenotypes in F_2				Test for 9:3:3:1	
	Tall, normal	Tall, zigzag	Dwarf, normal	Dwarf, zigzag	x^2	P
1. NJ863070 X PI227224	117	6	7	33	94.40	≈ 0
2. D76-1609 X PI227224	318	26	18	106	311.70	≈ 0
Pooled	435.	32	25	139	406.00	≈ 0
Heterogeneity					0.80	>0.50

SOYBEAN RESEARCH INSTITUTE
Nanjing Agricultural University
Nanjing, 210095
The People's Republic of China

Selection Effectiveness in a Recent Constructed Random-mated Soybean Population Possessing *ms1* Gene for Genetic Male Sterility.

Introgression of germplasm from different regions made both accumulation of favorable genes and complement of desirable traits available, combinations of high yield varieties from Northern China (NC) and Huang Hauai Hai Valley (HHH) and high protein content varieties from Southern China (SC) would make joint selection of yield and protein possible, thus to improve breeding efficiency.

There were two ways for making introgression between great number of genetic resources, one was by hand pollination, another was by open pollination through male sterile (MS) genes. Since large number of pollination by hand for soybean was difficult, thus the most convenient way was to use MS gene for cross pollination in the process of recurrent selection. Burton, et al. (1983, 1990) reported recurrent selection of yield and oleic acid percentage in oil. Song, et al. (1993) also reported a base population with high seed yield and high protein content consisting of forty varieties (lines) from SC, HHH and NC by the method, the work paved the way to the recurrent selection. However, in the process of selection, whether selection was to be made on the basis of MS plants in the segregation generation or of the progenies of male-fertile (MF)plants was still a problem, moreover, if selection was to be made on the progenies of the MF plants, the accuracy of selection would be affected by MS plants varied from progenies to progenies in two thirds of MF derived progenies, although theoretically, one third of MS plants were be showed up in each progeny. Furthermore, as tested progenies were increased, more field work was to be involved, therefore, the principal objective of the research reported herein was to determine the effectiveness of MF individual plant selection and visual selection based on progenies.

Synthesis of Population

Sixteen varieties with high protein content (over 45%) from SC, four with high yield from HHH were mated to a *ms1* MS maintainer line, N69-2774 (pollen donors), the F1 plants of twenty crosses were mated to other six varieties with high yield from NC, twelve varieties with high yield from HHH, two varieties with high yield and high protein content from SC respectively in 1989, the varieties and mating were listed in Table 1 and Table 2. Plants were selfed in 1991, twenty seeds from each cross were sampled to form one set, four hundred sets were formed altogether, they were sowed in dual dates with fifteen days interval to ensure random mating, seeds from MS

plants were harvested. In 1993, the seeds were mix planted, at maturity, plants were harvested according to their mature dates, plants matured before Oct. 1, and after Oct. 25 formed early population and late population, two populations were further divided into two and three sub-populations, designated as E1, E2, L1, L2 and L3 according to their growing length. Eighty plants from E1 and E2, fifty plants from L1, L2, and L3 were selected and observed for the seed yield and seed components in 1993, each plant developed into single progeny and planted in a single row with four meter length, one half meter row spacing in 1994, altogether, there were three hundred and ten rows. At harvest, recorded the number of MF plants and measured the yield and components in each row.

Table 1. Varieties and their origins

Code	Varieties	Origins	Code	Varieties	Origins
p0	N69-2774#				
p1	Xinyigaoliuhongmaozi	HHH	p21	Sidou 11	HHH
p2	Tongshanliushuaitianedan	HHH	p22	Doujiao 66	HHH
p3	p1313	SC	p23	Huaidou 88-24	HHH
p4	Binhaiwuhuazuanding	HHH	p24	Houzimao	SC
p5	Gunyunpingdinghuanyi	HHH	p25	Zhongdou 19	SC
p6	Lianshuituolagui	HHH	p26	Zhongzuo 84-C-24	HHH
p7	Shuyangxiahezei	HHH	p27	Tie 8115-3-2	NE
p8	Guanyundahongmaodoujia	HHH	p28	Tiefeng 25	NE
p9	Huaiandahuangke	HHH	p29	Heinong 25	NE
p10	Tongshangniumaohuang	HHH	p30	Heinong 33	NE
p11	Donghaidahongmao	HHH	p31	Liaodou No. 4	NE
p12	Sihongqituhuang	HHH	p32	Tiefeng 21	NE
p13	p1615	SC	p33	Tiefeng 24	NE
p14	Nannong 467332	SC	p34	Tie 8224-7	NE
p15	Nannong 467123	SC	p35	Dongnong 38	NE
p16	Nannong 467085	SC	p36	Liaodou No. 3	NE
p17	Nannong 88-213	HHH	p37	Jiunong 17	NE
p18	Nannong 88-217	HHH	p38	Suinong No. 8	NE
p19	Nannong 88-218	HHH	p39	Nannong 87C-38	SC
p20	Huaidou No. 2	HHH	p40	Nannong 88-29	SC

Table 2. Matings in 1989 and in 1990

1989	1990	1989	1990
p1xp0	(P1xp0)xp21	p11xp0	(p11xp0)xp31
p2xp0	(P2xp0)xp22	p12xp0	(p12xp0)xp32
p3xp0	(P3xp0)xp23	p13xp0	(p13xp0)xp33
p4xp0	(P4xp0)xp24	p14xp0	(p14xp0)xp34
p5xp0	(P5xp0)xp25	p15xp0	(p15xp0)xp35
p6xp0	(P6xp0)xp26	p16xp0	(p16xp0)xp36
p7xp0	(P7xp0)xp27	p17xp0	(p17xp0)xp37
p8xp0	(P8xp0)xp28	p18xp0	(p18xp0)xp38
p9xp0	(P9xp0)xp29	p19xp0	(p19xp0)xp39
p10xp0	(P10xp0)xp30	p20xp0	(p20xp0)xp40

Results

1. Correlation between MF plants and its progenies.

As it was shown in Table 3, correlations of 100-seed weight between MF plants and its progenies were significant in E2, L2 and L3 sub-populations at the probability levels of 5% or 1%, correlation of seeds per pod was only significant in E2 sub-population, therefore, selection of yield and other yield components except 100-seed weight based on single MF plant in the segregation population was ineffective, possible reason for that was the plants were all heterozygous.

Table 3. Average yield, yield components and correlation between MF plants and its progenies in each sub-populations.

	Yield (g/plant)	Pods/plant	Seeds/pod	100 Seed weight
E1	18.2	59.9	1.80	17.4
r	.15	0.04	0.04	0.18
E2	18.0	55.9	1.8	17.0
r	-.08	-0.19	0.41**	0.22*
L1	14.1	41.20	1.80	19.7
r	0.15	-0.16	0.12	0.38**
L2	14.4	44.1	1.8	19.0
r	-0.05	-0.08	0.01	0.44**
L3	17.8	59.4	1.8	17.5
r	1.23	-0.27	0.22	0.27*

* denotes significant at 5% probability level.

** denotes significant at 5% probability level.

2. Effectiveness of visual selection.

Table 4. Average yield of 20% progenies with the highest scores and with the lowest scores in different sub-populations.

Sub-populations	Mean 1(g/plant) 20% best progenies	Mean 2 (g/plant) 20% worst progenies
E1	24.0	12.4
E2	25.4	11.4
L1	18.6	10.2
L2	20.0	9.5
L3	23.1	14.0

Table 5. Average yield per plant under each visual scores

Sub-population	Score 2	Score 3	Score 4	Score 5
E1	17.6	17.5	19.4	19.6
E2	16.4	16.2	20.6	
L1	13.8	14.2	17.0	
L2	14.4	14.9		
L3	17.6	18.9		

Three hundred and ten progenies planted in rows were evaluated in the field by four soybean breeders according to their experience, five scores were used for ranking, progenies with score 5 were the best, while with score 1 were the worst, progeny scores were averaged over four records, progenies were then harvested after the numbers of MF plants in each row had been recorded, results were given in Table 4 and Table 5. In Table 4, actual yield differences between 20% best lines and 20% worst lines classified by visual selection were great. In Table 5, it could be seen that as scores increased, yield in the rank also increased in most of the cases, therefore, visual selection could be useful in the process of recurrent selection, especially when MS gene was used to facilitate random mating and when population to be observed was large.

References

Burton, J. W., R. F. Wilson, and C. A. Brim. 1983. Recurrent selection in soybean. IV. Selection for increased oleic acid percentage in seed oil. Crop Sci. 23:744-747.

Burton, J. W., E. M. K. Koinange, and C. A. Brim. 1990. Recurrent selfed progeny selection for yield in soybean using genetic male-sterility. Crop Sci. 30(6):122-126.

Song Qijian, Wu Tianxia, Yang De, Gai Junyi, and Ma Yuhua. 1993. Synthesis of a population with high protein content and high yield in soybean. Acta Agriculturae Sinica No. 1:164-167.

Song Qijian
Wu Tianxia
Gai Junyi
Qiu Jiashen

MENDELEUM UNIVERSITY OF AGRICULTURE AND FORESTRY
Faculty of Horticulture
Brno CZ-691 44 Lednice na Morave
Czech Republic

Identification of Soybean Cultivars Through Isozymes

Using methods shown in Kadlec et al. (1994 a, b) and Létal and Kadlec (1995), the following results have been achieved concerning the enzymatic markers of soybean:

1. Test of association between marker loci Pb/pb (sharp/blunt trichome terminations) and group of QTLs (Table. 1) was significant ($P < 0.05$) only for number of leaves.

2. Test of linkage disequillibrium between QTLs and loci Ep/ep (high/low activity of peroxidase seed coat) - see Table. 2 - was not significant ($P < 0.05$). It means that within the scope of sample lines the difference between the group with dominant allele and the group of homozygous lines with recessive allele is not statistically significant for any of quantitative traits observed. Only for a flowering period the significant level $P=0.1$ seems to prove significance in the case of a higher number of treatments.

3. A significance of linkage disequillibrium was not found in the case of loci N/n (an 'eye' is present or not present) and Ep/ep - see Table. 3. On the other hand the significance could be achieved between genotypes with loci Ep and N.

4. During selection of prospective cultivars two lines with unique isoenzyme systems have been found (Table. 4): the line 'L-059' carrying s/vha band (slow/very high activity) for URE and the line 'L-098' carrying probably 'null' allele for AMY (null allele for b-amylase) as the activity of anodic migrating isozymes was not proved. Both lines are in our disposal and could be available on demand.

5. For a high activity the systems ACP, AMY, PGM, SOD and URE have appeared as unfailing identificators of hybrid seeds in F1 generation (through micro-sample) - see Table. 5

Table 1: Test of association between marker locus Pb/pb and quantitative traits

Trait	Phenotype classes		t-test		Mann-Whitney
	Pb	pb	t	P	test
Weight of plant [g]	89.3	102.7	1.25	1	0.47
Height of plant [cm]	78.5	83.1	1.38	0.17	0.28
Number of branches	5.7	5.9	0.74	0.46	0.87
Number of internodes	15.8	1.29	0.20	0.36	1
Number of pods	79.3	89.8	1.24	0.22	0.34
Number of seeds	149.5	170.2	1.25	0.21	0.35
Weight of pods [g]	28.1	33.3	1.71	0.09	0.11
Number of leaves	37.7	59.7	2.19	0.03*	0.09
Weight of leaves [g]	41.2	58.4	1.73	0.09	0.19

* $P < 0.05$

Table 2: Tests of linkage disequillibrium between quantitative traits observed and loci Ep/ep (high/low activity of peroxidase seed coat)

Trait	N	Ep		ep		t	P
		mean	st. dev.	mean	st. dev.		
Shape of seeds	43	2.95	0.05	2.90	0.07	0.65	0.52
Length of seeds	43	7.73	0.05	7.81	0.07	-0.86	0.39
Width of seeds	43	6.53	0.04	6.61	0.05	0.61	0.55
Length of middle leaf	28	10.11	0.24	10.30	0.19	-1.14	0.25
Width of middle leaf	28	6.72	0.18	6.44	0.17	1.15	0.26
Length of leaf petiole	28	15.51	0.67	14.97	0.51	0.63	0.53
Number of seeds	42	31.24	1.95	30.62	1.76	0.24	0.81
Number of pods	42	18.12	1.15	16.84	0.88	0.88	0.38
Number of seeds/pod	42	1.76	0.04	1.80	0.04	-0.65	0.52
Period to germination	43	17.83	0.71	18.55	0.74	-0.70	0.49
Period to flowering	43	41.19	0.69	40.75	0.98	0.41	0.68
Period to maturity	43	129.44	1.76	123.93	2.83	1.68	0.10

* $P < 0.05$

Table 3: Test of linkage disequillibrium between loci N ('eye' is not present) and Ep/ep (high/low activity of peroxidase seed coat)

Locus N	Locus Ep/ep		Test
	Ep	ep	2.73
'Eye' is present	4	9	P = 0.098
'Eye' is not present	39	31	P = 0.133

Table 4: Identification of new cultivars through isozymes

	Enzymatic systems				
Cultivar	ACP	AMY	PGM	SOD	URE
L-059	s	s	s	a/b	s/vha
L-098	s	null	s	b/b	s

Table 5: Paternity test (hybrid constitution of the seed) in F1 generation

		Enzymatic systems				
Genotype	pod/seed	ACP	AMY	PGM	SOD	URE
L-059		s	s	s	a/b	s/vha
L-053		s	f	f	a/b	s
L-059 x L-053	35/7	s	f/s	f/s	a/b	s

References

Kadlec, M., J. Létal, J. Stejskal, M. A. Griga, J. Goliás. 1994a. Identification of soybean cultivars using isozyme electrophoresis. Soybean Genetics Newsletter. Vol. 19:101-110.

Kadlec, M., J. A. Létal, B. Janousek, 1994b. Screening morphologic and biochemical markers of Glycine species. Zahradnictví, 21 (3):201-211.

Kadlec, M., J. Létal. 1995 New Soybean Cultivars Selected in Mendeleum for the Region of Southern Moravia. Soybean Genetics Newsletter. Vol. 20.

Létal, J., M. Kadlec. 1995 Association between Morphological Characteristics in F2 Populations of Soybean. Soybean Genetics Newsletter. Vol.20.

Kadlec Miroslav

Létal Jiri

AGRICULTURAL BOTANY DEPARTMENT
College of Agriculture
Kolhapur-416004
(M.S.), India

Biomass Partitioning and Growth Characters in Relation to Plant Architecture in Soybean

Abstract

A survey of biomass partitioning and growth characters in connection with yield was done on 41 soybean. Highest amount of biomass (129.0g) noticed in GS 80/G 76-3 was not associated with satisfactory yield. The low yield (13.42g)) of this strain was due to heavy deposition (52%) into stem. Genotypes ACPS 9023 and DS-7 showed heavy deposition into leaves and roots. The population means indicated that soybean plant has a tendency to deposit 19.77, 8.05, 29.42, 30.85, and 11.41 percent assimilates into grain pod wall, stems, leaves, and roots respectively. The highest yielding PK 472 showed a pattern of 28.73, 11.14, 27.44, 25.26, and 7.46 percent allocation in grain, pod wall, stem, leaves, and roots respectively. The correlations indicated the nature of communication and interactions among different traits. On the basis of correlations, genetic parameters and allocation pattern a plant with 30-35 cm height, 5-6 branches, 20-25 functioning leaves at physiological maturity, 110-115 d maturity period, and allocation pattern of 30, 10, 25, 25, and 10 percent of assimilates into grains, pod wall, stem, leaves, and roots respectively was desirable.

Introduction

Soybean (*Glycine max*, L. Merrill) is an important agronomic crop. Majority of the existing cultivars of this crop are derived from limited parental lines. The attempts on designing efficient plant architecture in this crop need a greater emphasis to exploit it's yield to a fullest extent. The breakthrough in cereal yields has been achieved through the development of such efficient plant types. Looking to the aggressive's vegetative growth, balanced partitioning appears to be important criterion in designing a plant architecture in grain legumes. Current investigation is aimed at proposing a plant architecture on the basis of pattern of allocation of assimilates in grain, pod wall, stem, leaves, and roots in association with the growth characters like height, branches, leaves and maturity period.

Materials and Methods

A population of 41 entries of soybean was raised in randomized block design with two replications during rainy season of 1995 at

College of Agriculture, Kolhapur, Maharashtra (India). The sowing was done at a spacing of 45.0 cm in a plot size of 3.60 x 5.0 mtr. Observations on height, number of branches, number of leaves, dry matter of stem, leaves, roots, grains, and pod wall were recorded for each genotype at physiological maturity. Harvest index was estimated according to formula given by Donald and Hamblin (1976). Genetic parameters for quantitative traits were worked out according to formulae given by Singh and Chaudhari (1977).

Results and Discussion

Data on 10 genotypes from a base population of 42 soybean entries showing extreme points of range of different components of biomass p[partitioning are given in Table 1. Adequately high biomass is pre-requisite for formation of fairly high yield. In current investigation GS 80/G/76-3 produced highest biomass (129 g), but showed hardly 10.32 percent of it's allocation into grain yield (13.40 g) which is not even equal to population mean for yield (14.89 g) In contrast ACPS 92055 with poorest biomass production (48.79 g) allocated 19.38 percent assimilates into grain. This clearly indicate that not merely high biomass formation but it's greater allocation towards grain is more important. Biomass is the total organic framework from which different parts derive assimilates for the growth mostly in a competitive way. Critical perusal indicated that the genotype GS 80/G/76-3 has invested more than 52 percent assimilates into stem as against the population mean of 29.42 for stem weight percentage. The genotypes ACPS 9023, DS-7, showed high assimilate depositions into leaves (45.78%) and roots (16-88 percent) respectively. Aggressive growth of stems, and leaves is regarded as a mechanism of adaptation under unfavorable or stress condition in grain legumes which generally occurs at the cost of low yields. In this connection it must be noted that in shorter modern varieties high grain yield have been achieved at the expense of stem weight per unit area (Hay and Walkar, 1989).

High harvest index has remained an important criterion in many breeding programs (Donald and Hamblin, 1976; Gifford et al., 1984). In current investigation HI ranged widely (11.17 to 31.03 percent) indicating a good scope for the selection of this efficiency trait. However, it is equally true that high yield will follow if maximum of dry matter is directed to harvested portion so long as roots, stems and leaves are not starved to limit the extent that their functioning limits the productivity. This warrants that a balanced partitioning of assimilates into grain, leaves, stems and roots constitutes a basic requirement in designing a plant architecture for high grain yield. The population means for percent allocation indicated that in soybean there is a tendency to allocate 19.77, 8.05, 29.42, 30.85, and 11.41 percent of assimilates into grains, pod walls, stem, leaves, and roots respectively. It is difficult to propose ideal pattern of allocation. However, the one noticed in pk 472 appears to be characteristically responsible for it's highest yield performance (29 g/plant). The percent allocation in grains, pod wall, stem, leaves and roots in this genotype was 28.73, 11.14, 27.44, 25.26, and 7.46 percent respectively with a total biomass structure of 100.95 g/plant.

On the background of allocation pattern of population mean basis and the one noticed in PK 472 it can be proposed that it would be desirable to isolate genotype with allocation pattern of 30, 10, 25, 25, and 10 percent in grain, pod wall, stem, leaves, and root respectively.

Achieving such a desired kind of allocation pattern is rather difficult as the growing or metabolically active parts compete with each other for circulating growth material. According to Trewavas (1985) various plant parts interact with each other and these interactions are evidences of internal communications in the plant body. The correlation coefficients indicate the nature and extent of such communications of interactions among different plant parts. From correlation table (Table 2) it is revealed that genotypes with profuse branching, more number of leaves and long maturity period posses high yielding ability. Total biomass, stem weight, plant height and pods per plant were not correlated with yield, but had positive correlation with number of leaves and branches per plant indicating their indirect impact on yield. Root weight showed significant positive correlation with stem weight. number of pods and maturity period which indicated that long duration types have heavy stems which are supported by stronger roots resulting into profuse number of pods per plant. Foliage weight showed positive correlation only with total biomass. Plant height showed positive correlation with stem weight, number of leaves, branches, and pods per plant. Importantly these are the genotypic correlations and hence have a distinct importance as they indicate genetic control of the 'Communication' in respect of partitioning and morphogenesis. The proposal of ideal partitioning and plant architecture would therefore carry a sort of greater validity.

From Table 3 it is revealed that the maturity period had a range of 90.5 to 117 days with a population mean of 101 days. This being highly correlated with yield and having high heritability, a search for short duration would lead to low yield. A period of 112 days noticed in the highest yielding type PK 472 appears to be proper to provide necessary opportunity for adequate biomass formation, differentiation, and allocation at different sites. Further, the number of branches inspite of it's high positive correlation with yield appeared to be lowly heritable and had very low genotypic variability. It is therefore difficult to improve it. It's high correlation with highly heritable maturity period however, provides a scope for indirect selection. This selection needs to be done with due cautionsness as higher branching is likely to deposit heavy allocation into stem due to their strong positive correlation. In this connection approximately six branches as noticed in PK 472 would remain an ideal number.

Adequate number of functioning leaves is necessary during critical period of pod development in soybean. A maximum 35.66 leaves have been noticed in current investigation. Excessive foliage is however undesirable for many reasons including heavy transpirational losses in 'water spender' crop like soybean (Chatterjee and Bhattacharya, 1986) leading to water stress in the critical phase. Around 20 to 25 functioning trifoliate leaves would serve the

purpose of adequate assimilate supply to reproductive sink.

Plant height derives a foremost attention in deciding an efficient plant architecture. In current investigation a range of 17.16 to 113.17 cm for this character is noticed. Tall, viny types are generally prone to lodging and hence not desirable. Very short types would be unable to produce adequate number of leaves, branches and pods. An intermediate height of 30-40 cm even less than population mean would be desirable from crop geometry and management point of view. This character being highly heritable with high genetic advance appeared to be under control of additive genes.

On the basis of wide variability at genotypic as well as phenotypic levels, nature and extent of character association, pattern of allocation into different parts and genetic parameters of various characters, it is possible to propose an efficient plant architecture in soybean. A type with 30 to 35 cm height, 20-25 leaves at pod development, 5-6 branches, a maturity period of 110 to 115 days, and an allocation pattern of 30, 10, 25, 25, and 10 percent in grains, pod wall, stem, leaves, and roots respectively with a total biomass of 100 g/plant would be desirable. Standardization of crop geometry and agrotechnique would be able to exploit this plant type efficiently.

References

Chatterji, B. N. and K. K. Bhattacharya. 1986. Physiology of grain legumes. Principles and Practices of Grain Legume Production. pp. 51-143. Oxford and IBH Publishing Co., New Delhi.

Donald, C. M. and J. Hamblim 1976. Biological yield and harvest index of cereals as agronomic and plant breeding criteria. Advances in Agronomy. 28:361-405.

Gifford, R. M., J. H. Thorne, W. D. Hitz, and R. T. Giaquinta. 1984. Crop productivity and photoassimilate partitioning. Science. 225:801-8.

Hay, R. K. M. and M. J. Walker. 1989. The importance of the harvest index. An Introduction to the Physiology of Crop Yield. pp. 119-123. Longman, Scientific and Technical Co-published in the United States with John Wiley and Sons, New York.

Singh, R. K. and B. D. Chaudhari. 1977. Biometrical methods in quantitative genetic analysis. Kalyani Publisher, New Delhi-Ludhiana.

Trewavas, A. 1985. A pivotal role for nitrate and leaf growth in plant development. In Baker, N. R., W. J. Davis, and C. K. Ond (Ed.). Control of leaf growth pp. 77-91. Cambridge University Press, Cambridge

S. S. Mehetre

B.M. Jamadagni

Table 1. Genotypes showing extreme points of range for biomass partitioning in soybean.

Genotype	Grain Yield (G/pl.)	Harvest Index (%)	Bio-logical yield (g/pl.)	Stem g/pl.	Leaves g/pl.	Roots g/pl.	Pod wall	Per cent allocation				
								Grains	Pod wall	Stem	Leaves	Roots
PK 472	29	31.03	93.45	27.70	25.50	7.5	11.25	28.73	11.14	27.44	25.26	7.43
GS 80/G76-3	13.40	11.17	129	68.35	28.70	9.80	9.45	10.32	7.29	52.70	22.13	7.56
ACPS 92-27	18.00	22.84	78.80	19.70	35.20	8.65	5.9	20.58	6.75	22.53	40.25	9.89
DS 39	14.82	14.71	100 .72	43.30	35.2	13.8	7.4	12.94	6.45	37.81	30.74	12.05
DS 97	28.00	23.88	117.25	56.70	22.75	9.15	9.8	22.15	7.15	44.86	18.00	7.24
ACPS 90216	9.45	16.26	58.1	18.40	26.45	5.9	3.8	14.76	5.94	28.70	41.33	9.21
ACPS 92055	10.70	21.97	48.7	15.60	18.80	6.5	3.6	19.38	6.52	28.26	34.06	11.78
ACPS 90231	9.85	19.44	50.65	11.90	25.80	5.7	3.1	17.48	5.50	21.11	45.78	10.13
DS 7	10.30	20.21	50.95	24.80	8.30	10.35	3.2	16.81	8.48	40.46	17.37	16.88
DS 51	19.55	27.71	70.55	19.60	25.10	4.80	6.3	25.95	8.36	26.01	33.21	6.37
Sample Mean Population Mean	14.89	22.38	67.12	23.21	22.92	8.51	6.08	19.77	8.05	29.42	30.85	11.41

Table 2. Genotypic correlation coefficients for biomass partitioning and growth characters in soybean

	Stem weight	Foliage weight	Root weight	Leaves per pl.	Branches	Height	Pods	Duration	Yield
Biomass	0.776**	0.479**	0.274	0.377*	0.737**	0.295	0.323*	0.933**	0.309
Stem wt.	-	0.262	0.367*	0.338*	0.725**	0.510**	0.206*	0.595**	0.281
Foliage wt.		-	-0.081	-0.108	-0.263	0.207	-0.056	0.171	-0.005
Root wt.			-	0.191	0.265	0.159	0.394**	0.924**	0.286
Leaves				-	0.540**	0.559**	0.754**	0.190	0.435**
Branches					-	0.725**	0.673**	0.423**	0.681**
Height						-	0.450**	0.130	-0.163
Pods							-	0.361**	0.226
Duration								-	0.517**
Yield									

Table 3. Statistical and genetic parameters of some growth attributes in soybean

	Plant Height (cm)	Branches/ pl.	No. of leaves plant	Pods/ Pl.	Maturity period (days)	Root weight (g/pl.)	Stem weight g/pl.	Wt. of leaves g/pl.	Grain yield g/pl.	Pod wall wt.
Mean	61	4.36	15.26	61.07	101	8.51	23.21	22.95	14.88	
Maximum	113.17	9.99	35.66	98.66	117	13.80	68.35	35.20	29.00	
Minimum	17.16	2.99	7.66	41.03	90.5	4.80	11.90	8.30	9.45	
GCV %	33.09	3.97	33.71	25.06	7.07	24.08	45.51	22.48	30.95	
PCV %	35.41	32.59	43.17	29.82	7.17	27.30	46.51.	26.75	34.46	
h^2 (b)	87.30	17.0	61.00	70.60	97.70	77.60	91.80	70.60	80.70	
Genetic Advance	38.86	0.5	8.25	26.48	14.55	3.72	20.67	8.92	8.53	

MAHATMA PHULE AGRICULTURE UNIVERSITY
Agricultural Botany Section
College of Agriculture
Vidyanagar, Kolhapur-416 004
India.

Gamma Ray Induced High Oil Content in Soybean (*Gycine max* (L.) Merrill.)

Recent spurt in Soy's popularity in India is due to the commercial value of its various by-products as well as number of advantages associated with soybean oil e.g. reducing heart diseases, gout and rheumatism. Soybean research and development in the recent past resulted in alarming growth of soybean cultivation.

Development of varieties relatively from linolenic acid, lipoxygenase enzymes and trypsin inhibitors etc. are considered to be major steps in soybean improvement, besides striving for better seed germinability and minimizing pod shattering (Prasad, 1974).

Defect rectification in soybean varieties for their improvement has been possible through mutagenesis (Bhatnagar and Tiwari, 1969). Since a negative association between oil and protein is well established, we attempted mutation breeding program in soybean with a view to combine high oil and seed yield in existing well adapted genotypes.

Since micromutations are affecting economically important traits they are of great importance to plant breeders. Dry seed of the varieties were treated with gamma ray doses (10, 20, 30 ,40, and 50 kr). In M_2 and M_3 generations both micro and macro mutations were obtained. Single plant from M_2 generation were selected discarding argonomically undesirable plants and their progenies were evaluated in M_4 generation. Oil percentage was determined by MMR permitting non-destructive estimation in seeds. In all, 75 families were evaluated for their yield performance and oil content. All of them showed greater variability, increased variance and extended range; for oil content. When compared with the respective unirradiated parents as control, the analysis of variance showed significant differences amongst the genotypes for all the characters indicating the presence of substantial genetic variability. Genetic parameters of variation for yield and its components are given in Table 1. The highest genetic variation was observed for pods/plant followed by plant height and yield/plant. High heritability coupled with high genetic advance observed for 100-grain weight, while moderate heritability coupled with high genetic advance observed for the character. Branches/plant and pods/plant indicating these characters respond to selection effectively. M_4 generation showed variability for oil content to the extent of 26.47 with expected genetic advance 8.58%. Oil content was found to be highly

significant correlated with days to maturity (0.56), branches per plant (.0.34), while it was a negatively correlated with plant height (-0.38). Yield per plant showed a negative significant correlation with oil content (-0.29) (Table 1.)

From the M_4 generation, only nine families showing significantly higher yield and oil content were selected and their performance was evaluated along with their unirradiated parents as controlled checks and with other standard checks also. Amongst these nine mutants; (Table 2.) ACK-S-93001 (2527 kg/ha) gave highest yield and followed by ACK -S-93003 (2475 kg/ha) and ACK-S- 93012 (2387kg/ha) over highest yielding check ACK-S-1 (2323 kg/ha).

The grain yield was observed highly and significantly correlated with 100-grain weight, branches/plant and pods/plant. Branches/plant and 100-grain weight had maximum positive direct effect on grain yield. The character days to maturity had negative correlation with yield but had positive direct effect on yield.

From the above studies it is revealed that branched/plant, 100-grain weight, pods/plant and days to maturity are most important yield contributing characters which should be given due importance in selection for improvement in yield.

As regards oil content all, mutants showed increased oil content over their parents. Highest oil content was (24.0%) recorded in ACK-S-93005 followed by ACK-S-93012 (23.2%), but both these mutants showed lower yield, which might be because of significant negative association observed between yield and oil content. It was found to be disassociated with some extent in mutant ACK-S-93001 in which both oil content (22.09%) and yield (2527 kg/ha) found to be increased over its parent ACK-S-2 for oil (19.9%) and seed yield (2210 Kg/ha).

Results reported here indicated that rectification of defect of negative association between oil and yield can be achieved by mutation breeding which is in conformity with earlier reports (Bhatnagar and Tiwari, 1969; Bhatnagar et al., 1993 and Cogner et al. 1976).

References

Bhatnagar, P. S. and P. S. Tiwari. 1991. Soybean improvement through mutation breeding in India. Plant Mutation Breeding for crop improvement. Vol. 1. IAEA, Vienna, pp. 381-391.

Bhatnagar, P. S., S. P. Tiwari, and C. Singh. 1992. Disrupting the negative association between oil and protein content in soybean seeds through mutagenesis. Mut. Breed. New. Lett. Vol. 37: 7.

Cogner, B. V., L.W. Skinner and L.N. Skold. 1976. Variability for components of yield induced in soybean by seed treatment with gamma radiation, fission neutrons and ethylmethyl sulphonate. Crop. Sci. 16: 233-236.

Prasad, M.V. R. 1994. Oil seeds growth should accelerate. Hindu Survey of Indian Agriculture. pp. 50-53.

S. S. Mehetre
C. R. Mahajan
R. B. Shinde
P. M. Dhumal

Table 1. Genetic parameter of Variation in gamma irradiated M_4 generations of Soybean (*Glycine max*, L.) Varieties

Characters/Parameters		Days required for 50% Flowering	Days required for Maturity	Plant height (cm)	100 grain weight (gm)	Pods/ Plant (Nos.)	Branches/Plant (Nos.)	Oil %	Yield/Plant (gm)
I. Analysis of Variance :									
M.S.S.	D.F.								
a) Replication	1	81.56	416.25	2127.50	29.63	8622.50	1.04	13.82	86.03
b) Genotypes	74	159.10**	588.75*	2356.16**	75.49**	8678.8*	15.54*	31.94*	779.18
c) Error	74	7.88	106.74	968.70	9.66	3321.70	6.00	18.67	89.52
II. Genetic Parameters :									
1) Mean		53.75	108.93	62.90	11.96	100.82	4.54	23.08	11.30
2) C.V. %		1.65	3.00	15.65	8.22	18.77	17.06	26.47	5.92
3) Coefficient of Variability (P)		7.56	22.60	69.35	3.29	267.86	0.48	0.66	34.48
4) Coefficient of Variability (G)		8.35	33.27	66.22	4.26	60.03	1.08	2.53	43.44
5) Heritability (h^2) %		90.60	67.90	41.70	77.30	44.60	44.30	26.20	79.40
6) Genetic Advance (G.A.)		5.39	8.07	11.08	3.29	22.51	0.95	8.58	10.78
7) Expected G. A. (% mean)		10.03	7.41	17.61	27.48	22.32	40.85	3.72	95.39
III. Coefficient of Correlation and Path analysis :									
1) Days required for 50% flowering	rg^b	1.00	0.59**	0.18	-0.57**	-0.52**	-0.27*	0.13	-0.37**
	pc	1.21	-1.38	-0.23	0.28	-1.12	0.73	0.16	-
2) Day required for maturity	rg^b	-	1.00	0.17	-0.76**	-0.42**	-0.41**	0.56**	-0.67**
	pc	-2.23	0.72	0.21	0.36	-0.91	1.10	0.67	-
3) Plant height (cm)	rg^b	-	-	1.00	0.52**	-0.24*	-0.65**	-0.38**	-0.39**
	pc	-1.25	0.22	-0.40	0.25	-0.52	1.76	-0.46	-
4) 100-grain weight (gm)	rg^b	-	-	-	1.00	0.51**	0.57**	-0.13	0.67**
	pc	-0.49	-0.69	1.77	0.65	1.11	-1.52	-0.15	-
5) Pods/Plant	rg^b	-	-	-	-	1.00	0.82**	0.02	0.40**
	pc	2.17	-0.63	0.98	0.30	-0.25	-2.19	0.02	-
6) Branches/Plant	rg^b	-	-	-	-	-	1.00	0.34**	0.64**
	pc	-2.69	-0.33	0.95	0.82	-0.28	1.77	0.40	-
Oil%	rg^b	-	-	-	-	-	-	1.00	-0.29*
	pc	1.19	0.16	-1.31	0.48	0.06	0.04	-0.90	-
8) Yield /Plant (gm)	rg^b	-	-	-	-	-	-	-	1.00

Residual effect = 1.062; G= genotypic; P=Phenotypic; rg^b=genotypic correlation coefficient; pc=Path coefficient;
Underline figures denotes direct effect; * and ** = significant at 1 and 5% level respectively.

Table 2. Field performance (Average of three locations) of promising high yielding mutants with high oil content grown during 1994-95 under varied agro-climatic conditions.

Sr. No.	Mutant No.	Parent variety	Gamma dose (kr)	Seed yield kg/ha				Days required for		Plant height (cm)	Pods/plant (No.)	Branches/plant (No.)	100 grain wt (gm)	Oil %	Leaf spot reaction
				L-I	L-II	L-III	Avg.	50% Flowering	Maturity						
1.	ACK-93001	ACK-S-2	30	3889	2111	1511	2527	51.00	106.67	51.11	44.67	4.28	12.45	22.9	R
2.	ACK-93002	ACK-S-2	30	3194	2056	1476	2242	48.55	100.22	49.75	49.54	4.71	11.70	20.6	MR
3.	ACK-93003	ACK-S-1	20	3263	2005	1604	2475	48.55	98.55	44.58	50.98	4.45	11.45	21.6	MR
4.	ACK-93004	ACK-S-1	30	2869	2032	1754	2217	48.66	99.00	54.94	50.23	4.36	11.35	22.6	S
5.	ACK-93005	ACK-S-1	30	2824	1671	1732	2073	48.66	101.44	48.82	52.94	4.54	10.55	24.0	S
6.	ACK-93007	ACK-S-2	30	2824	1833	1685	2114	49.11	103.33	50.31	48.76	4.51	11.40	22.6	S
7.	ACK-93008	ACK-S-2	30	3056	1824	1554	2150	50.11	99.00	48.38	51.49	4.90	11.20	21.5	R
8.	ACK-93012	ACK-S-3	30	3217	2275	1667	2387	50.33	98.66	48.04	50.48	4.39	12.25	23.2	MR
9.	ACK-93014	ACK-S-3	30	2661	1495	2484	2250	54.66	110.66	49.71	43.60	5.15	11.55	22.9	S
10.	ACK-S-1 check	Control	UR	3008	1653	2309	2323	52.66	106.66	49.73	44.50	4.06	12.25	21.8	MS
11.	ACK-S-2 check	Control	UR	2522	1861	1944	2110	50.00	104.66	61.96	36.44	3.53	11.40	19.9	S
12.	ACK-S-3	Control	UR	3056	1648	1736	2144	47.66	109.66	43.70	39.11	4.16	12.20	20.8	MS
13.	Pk-472 std.	check	UR	3101	1958	1668	2243	47.00	109.00	30.13	44.01	4.61	13.95	20.6	S
SE+ Kg/ha		-	-	286	123	82	'L' = Location; UR = Unirradiated; Std. = Standard; wt = Weight.								
CD 5%		-	-	NS	484	249									
CV		-	-	1619	1108	785									

L_1 -Kolhapur, L_2 - Gadhinglaj, and L_3 - Pune.

NATIONAL RESEARCH CENTRE FOR SOYBEAN (ICAR)
KHANDWA ROAD, INDORE-452001 (M.P.), INDIA

Screening of Indian Soybean Genotypes for Seed Longevity as Affected by Field Weathering

Soybean seeds are grouped in least storable category (Justice ans Bass, 1988). These seeds not only loose quality during storage but are also highly prone to field weathering, the process by which the quality of seeds deteriorates while seeds attain physiological maturity and subsequent ripening period in the field (TeKrony et al. 1980). The factors responsible for field weathering like high temperature and moisture both in the form of humidity and/or precipitation are more acute in subtropical and tropical countries (Paschal and Ellis, 1978). Such weather conditions are typical of major soybean growing areas of India. Bhatia et al. (1993) have reported very poor germination in the seeds collected at sowing time from the farmers field and this could be the possible reason of sub-optimal crop stand resulting in stagnant low national productivity (Bhatnagar and Karmakar, 1995). So far there has not been any effort to group the soybean genotypes for their response to field weathering. Therefore, the present study was an effort to screen the Indian soybean genotypes for their seed longevity as affected by field weathering.

Material and Methods

A replicated trial in plots of 3 rows x 6 m with 30 soybean genotypes was conducted during rainy season of 1994. Of the three rows, the middle row was harvested at harvest maturity, another was delayed harvested (after 15 days of harvest maturity) and the third one was delayed harvested after subjecting to daily sprays with water. The seed samples of each treatment were tested for seed viability by germinating at 25° C in an incubator for three days, The longevity of seeds was assessed by applying methanol stress test as described by Musgrave et al. (1980).

Result and Discussion

No significant difference in percent seed germination of 30 genotypes tested after normal and delayed harvesting were observed. The range was observed to be from 91%-100% (Table 1). The spraying of water along with delayed harvesting though resulted in significant differences but the germination was still quite high (83%-100%).

To obtain the desired plant population in the field, a minimum 80% germination in seed at the time of sowing is recommended (AOSCA, 1983). Byrd and Delousche (1971) reported that as seeds deteriorate during storage, there performance potential and vigor declines before there is any loss in viability (Standard germination). Thus the standard germination of seeds taken soon after the harvest may not accurately indicate the degree of seed deterioration. Hence, the methanol stress

test was performed to assess the seed longevity of these genotypes under different weathering treatments. The results revealed highly significant genotypic differences in percent seed germination. The germination was highly reduced in some of the genotypes and ranged from 56-100, 48-100, and 19-97% in the seeds collected at harvest maturity, delayed harvest and delayed harvest with daily water sprays, respectively. Even in seeds collected at harvest maturity resulted in loss of longevity and the degree of deterioration increased as harvesting was delayed and further couples with high moisture conditions created by spraying water on the standing crop.

The seed longevity in terms of germination percentage of 30 Indian soybean genotypes under worst weathering conditions created by delayed harvest coupled with water sprays is presented in Table 2. Only 50% of the genotypes tested showed desirable 80% germination. The genotypes showing better longevity along with high yields offer opportunity to be used in breeding programs aimed at improved seed longevity. Also, some of the India soybean genotypes are highly prone to field weathering. The delayed harvesting and occurrence of rains and high humidity at the time of maturity may further compound the problem of seed longevity resulting in poor plant population in field leading to low productivity.

References

AOSCA. 1983. Certification standards. Raleigh, NC.

Bhatia, V. S., S. P. Tiwari, O. P. Joshi, and N. A. Sharma. 1993. Effect of field weathering in soybean cv. Punjab 1 and JS 71-05. Seed Research 20:36-37.

Bhatnagar, P. S. and Karmakar, P. G. 1995. Achievements and prospects of breeding researches on soybean (Glycine max) in India. India Journal of Agricultural Sciences 65:1-9.

Bryd, H. W., and J. C. Delouche. 1971. Deterioration of soybean seed in storage. Proceedings of Association of Official Seed Analysts 61:41-57.

Justice, O. L., and L. H. Bass. 1978. Principles and practices of seed storage. USDA handbook, 506.

Musgrave, M. E., D. A. Priestley, and A. C. Leopold. 1980. Methanol stress test of seed vigour. Crop Science 20:626-630.

Paschal II, E. H., and M. A. Ellis. 1978. Variation in seed quality characteristic of tropical grown soybean. Crop Science 18:837-840.

TeKrony, D. M., D. B. Egli, and A. D. Phillips. 1980. Effect of field weathering on the viability and vigour of soybean seed. Agronomy Journal 22:749-753.

V. S. Bhatia

P.S. Bhatnagar

O. P. Joshi

Table 1. F-value, mean, range, standard deviation and coefficient of percent germination in the seeds of 30 soybean genotypes collected from varying weathering treatments.

Characters	F-value	Mean	Range	SD (+)	CV
1. Germination % after harvest					
Harvest maturity	2.97	97.7	92.0-100.0	2.85	2.92
Delayed harvest	1.00	96.8	90.7-100.0	2.92	3.02
Delayed harvest + water spray	4.62**	96.2	82.7-100.0	5.21	5.42
2. Germination % after methanol stress					
Harvest maturity	11.41**	90.4	56.0-100.0	12.45	13.78
Delayed harvest	24.56**	81.6	48.0-100.0	16.72	20.49
Delayed harvest + water spray	75.95**	74.3	18.7- 97.3	21.14	28.45

** Significant at 0.01 p

Table 2. Seed longevity in terms of percent germination of 30 soybean genotypes as affected by delayed harvest coupled with water sprayes.

Germination (%)	No. of genotypes	Genotype
0-20	1	NRC 7 (18.7)*
21-40	2	Pusa 40 (30.7), NRC 8 (38.7)
41-60	4	JS 71-05 (45.3), PK 262 (54.7), PK 416 (54.7), Bragg (58.7)
61-80	8	Hardee (61.3), Pusa 24 (62.7), PK 564 (68.0), PK 308 (69.3), PK 472 (70.7), MACS 124 (73.3), JS 80-21 (78.7), NRC 2 (80.0)
81-100	15	JS 335 (84.0), Monetta (85.3), NRC 1 (86.7), MACS 13 (88.0), KHSb 2 (88.1), JS 75-46 (89.3), MACS 58 (90.7), Pusa 20 (90.7), Pusa 16 (90.7), Punjab 1 (90.7), Gaurav (93.3), Pusa 22 (94.7), PK 327 (96.0), Kalitur (97.3), Durga (98.7)

* Values in paranthesis are percent germination after methanol stress test.

NATIONAL RESEARCH CENTRE FOR SOYBEAN
Indore (M. P.) India

Two New Weed-Host of Myrothecium roridum from India

During the routine survey for the diseases of soybean in Kharif 1995 at NRCS farm at Indore, a few weed plants were found having a leaf spot typical to Myrothecium leafspot of soybean.

The plants were collected and critically observed under the stereo as well as research microscope. The symptom appears as circular to oval tan colored spots encircled with dark brown colored margin, the centers are whitish to gray white in color with small pin size black color sporodochia, which comes out with rain or wind and form a hole (Fig. 1).

The spot was mounted in a cotton blue lacto-phenol stain and observed under research microscope. It was found M. roridum Tode ex Fries. Simultaneously the typical leafspot of soybean caused by M. roridum was also mounted for comparison. They were found absolutely the same.

These weed plants were identified as Corchorus atungulus (Fam. Tiliaceae) and Paronia sidaefolia (Fam. Malvaceae), which can work as potential source for harboring the primary inoculum of the disease. Hence, they must be eradicated from the field to reduce the inoculum potential and yield losses occurred due to this disease.

Perusal of literature (Sinha & Narain, 1992) revealed that these hosts have not been reported from India and form a new host record for the M. roridum.

Acknowledgement

Author is thankful to the Director, NRCS for providing the necessary facilities and encouragement and to Mr. S. S. Vasuniya for technical assistance.

References

Sinha, A. K. And U. Narain. 1992. A new host record of M. roridum from India. Indian Phytopath. 45(2):281.

M. M. Ansari

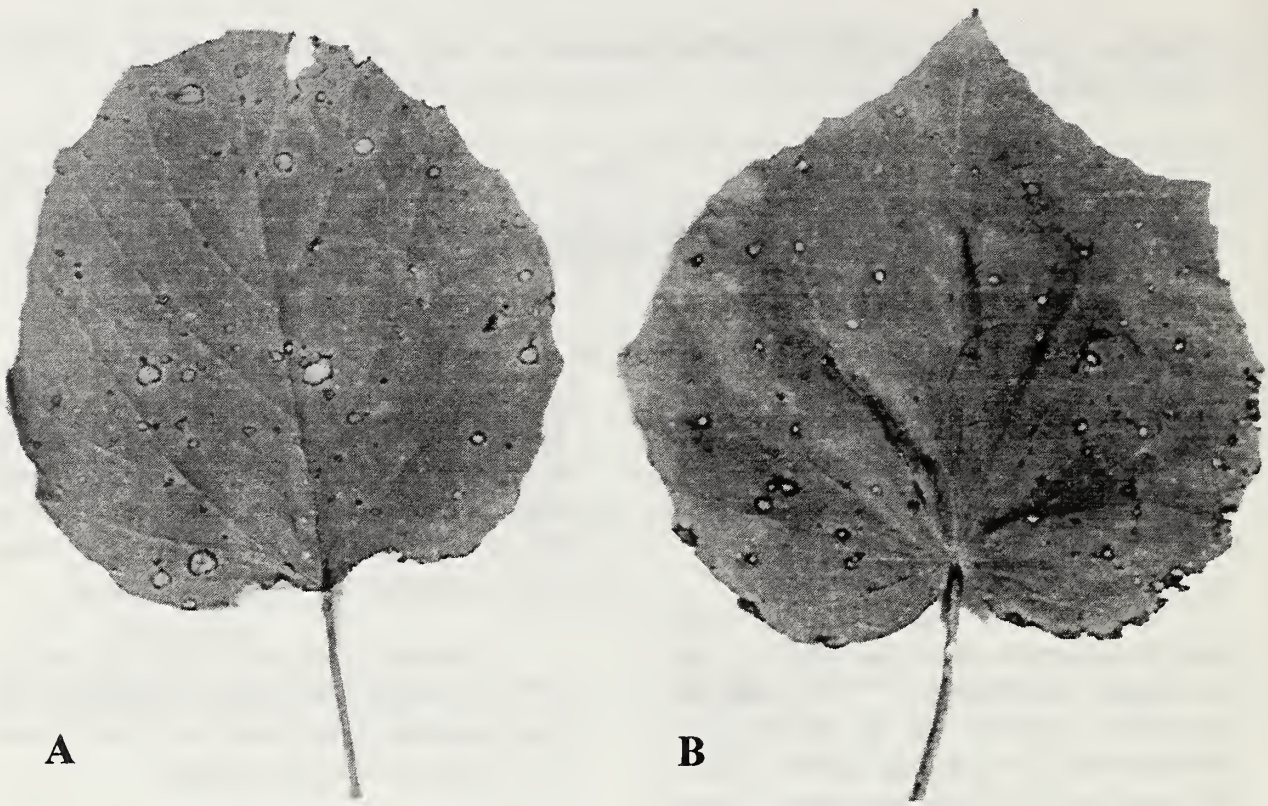


Figure 1.

A - Corchorus atungulus , B - Paronia sidaefolia showing Leafspot of M. roridum

HOKKAIDO UNIVERSITY
Faculty of Agriculture
Sapporo, 060, Japan

RFLPs of Chloroplast and Mitochondrial Genomes in Summer and Autumn Maturing Cultivar Groups of Soybean in Kyushu District of Japan.

Introduction

Two Japanese cultivar groups with different maturities of soybean (Glycine max (L.) Merrill), summer and autumn maturing cultivar groups, are cultivated consecutively in Kyushu, a southern district of Japan (Fukui and Arai, 1951; Nagata, 1959). Genetic constitution in the summer and autumn cultivar groups in Kyushu has been examined with isozymes and other biochemical and morphological genetic makers (Nagata, 1959; Hymowitz and Kaizuma, 1979; 1981; Hirata et al., 1994; 1996). These results indicate that the two cultivar groups have been differentiated through distinct phyletic lines. We evaluated polymorphisms of chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) in the summer and autumn cultivar groups in Kyushu by restriction fragment length polymorphism (RFLP).

Materials and methods

Tweenty four accession of the summer cultivar group and thirteen accessions of the autumn group were used in this study. Five to ten ug of total DNA isolated from leaf tissue was digested with four restriction endonucleases, BamHI, ClaI, EcoRI, and HindIII. After electrophoresis and southern blotting, digested DNA was hybridized with each of three probes, H2 clone (sugerbeet cpDNA, HindIII fragment, Kishima, 1988), atp6 (Oenothella spp. mtDNA, StyI fragment, Schuster and Brennick, 1987), and coxII 3' exon (sugerbeet mtDNA, SalI-EcoRI fragment, Senda et al., 1990). H2 clone contains the large single copy area near the juncture with the left member of the inverted repeat, where is a hot spot of structural variations in soybean chloroplast genome (Close et al., 1989).

Results and discussion

Table 1 shows RFLPs with H2 clone and two restriction endonucleases (EcoRI and ClaI). The 37 accessions tested were classified into three groups which corresponded with clastome group I to III of Close et al., (1989) (Shimamoto et al., 1992). Most of the accessions tested had chloroplast group I. All of the accessions of the summer cultivar group exhibited the chloroplast group I, whereas groups II and III were observed only in the autumn cultivars group. Close et al., (1989) found that chloroplast group I was predominant in cultivated soybean, whereas group III was observed only in 'Peking' and most of the G. soja accessions tested.

Table 1. RFLPs of cpDNA in summer and autumn cultivar groups in Kyushu district of Japan

Chloroplast group*	Probe Enzyme	H2 clone		Cultivar Group	
		EcoRI	ClaI (kbp)	Summer	Autumn
I		4.8	1.1, 2.4	24	8
II		4.8	3.5	0	1
III		2.5	3.5	0	4

* The classification of chloroplast groups followed Close et al. (1989).

Table 2. RFLPs of mt DNA in summer and autumn cultivar groups in Kyushu of Japan.

RFLP pattern no.	Probe Enzyme	coxII		atp6	Cultivar Group	
		HindIII	BamHI	BamHI (kbp)	Summer	Autumn
1		3.5	8.1	2.9, 5.0	18	8
2		3.5	8.1	5	6	1
3		8.5, 10	11, 15	5	0	4

The accessions tested, on the other hand, had tree RFLP patterns when analyzed with two mitochondrial probes (coxII and atp6) and two endonucleases (HindIII and BamHI) (Table 2). Two of the three RFLP patterns (no. 1 and 2) were observed in both summer and autumn cultivar groups. These patterns had the common fragments for coxII (3.5 kbp HindIII fragment and 8.1 kbp BamHI fragment), but showed different RFPLs for atp6 (2.9 kbp and %.0 kbp fragments and single 5.0 kbp fragment). No. 3 RFLP pattern was different from the two mentional above with RFLPs for coxII (8.5 kbp and 10 kbp HindIII fragments and 11 kbp and 15 kbp BamHI fragments), and was observed only in the autumn cultivar group. Grabau et al., (1989, 1992) and Hanlon and Grabau (1995) have classified soybean cultivars of the USA into eight groups by RFLPs with the 2.3 kbp mtDNA HindIII fragment and atp6 as probes. When analyzed with coxII and atp6 in our study, 'Minsoy' and 'Harasoy', which wre classified into the 'Bedford-type' of Grabau et al., (1992) and Lee et al., (1994), exhibited no. 1 RFLP pattern (3.5 kbp HindIII fragment and 8.1 kbp BamHI fragment for coxII, and 2.9 kbp and 5.0 kbp BamHI fragments for atp6), 'Biloxi' and 'Plametto' of the 'Arksoy-type' exhibited no. 2 RFLP pattern (3.5 kbp HindIII fragment and 8.1 kbp BamHI fragment for coxII, and 5.0 kbp BamHI fragment for atp6). It remains to be determined which of the eight groups classified by Grabau et al., (1989, 1992) and Hanlon and Grabau (1995) no. 3 RFLP pattern (8.5 kbp and 10 kbp HindIII fragment and 11 kbp and 15 kbp BamHI fragment for coxII, and 5.0 kbp BamHI fragment for atp6) corresponds to.

Table 3. Classification of summer and autumn cultivars in Kyush of Japan with RFLPs of cpDNA and mtDNA.

Chloroplast group	mtDNA RFLP pattern no.	Accession		
		Summer cultivar group		Autumn cultivar group
I	1	Aka ban	Kairyo shirome	Hakusui zairai 83a
		Amakusa	Kaneko	Ichinomiya zairai 83a
		Ao daizu	Kin	Itsuki zairai 83b
		Aochi	Matsuba daizu	Izumi zairai 83c
		Doyo mame	Misaki daizu	Oka daizu
		Hayakin	Natsu kurakake	Souta daizu
		Ichigo wase	Shimabara	Touyo zairai 83b
		Jin nai	Shin hounen	Zairai kuro daizu
		Kairyo gionbo	Wase kin	
I	2	Aka wase (00032980)		
		Kasuga zairai		
		Kisaya (1)		
		Matsuura		
		Sango wase		
II	2			Oushoku aki daizu
III	3			Aso zairai 83
				Ooita aki daizu
				Oukuchi zairai 83
				Shichijo zairai 83a

Table 3 shows combinations of RFLPs of cpDNA and mtDNA. All of the accessions classified into chloroplast group I showed the common RFLP pattern for mtDNA (3.5 kbp fragment with coxII/HindIII, 8.1 kbp fragment with coxII/BamHI, and 2.9 kbp and 5.0 kbp fragments with atp6/BamHI). However, the accessions with no. 2 mtDNA RFLP pattern (3.5 kbp fragment with coxII/HindIII, 8.1 kbp fragment with coxII/BamHI, and 5.0 kbp fragment with atp6/BamHI) were classified into two groups based on the chloroplast gruops. All of the summer accessions with no. 2 mtDNA RFLP pattern were classified into chloroplast group I, whereas 'Oushoku aki daizu' of the autumn group was categorized in chloroplast group II. All of the four accessions with no. 3 mtDNA RFLP pattern in the autumn cultivar group blonged to chloroplast group III. Because chloroplast group III awas predominant in G. soja distributed in Japan

(Shimamoto et el., 1992), the autumn cultivars with chloroplast group III and no. 3 mtDNA RFLP pattern might br derived from a hybrid between G. soja and G. max.

RFLP analysis of cpDNA and mtDNA this provids a powerful tool to trace the origins and disseminations of the summer and autumn cultivar groups in Kyushu. Studies on the diversity of organelle DNA are in progress for both cultivated and wild soybeans in our laboratory.

References

Close, P. S., R. C. Shoemaker, and P. Keim. 1989. Distribution od restriction site polymorphism within the chloroplast genome of the genus Glycine, subgenus Sojja. Theor. Appl. Genet. 77:768-776.

Fukui, S. and M. Arai. 1951. Ecological studies on Japanese soybean varieties. I. Classification of soybean varieties on the basis of the days from germination to blooming and from blooming to ripening with special reference to their geographical differentiation. Japan. J. Breed. 1:27-39. In Japanese.

Grabau, E. A., W. H. Davis, N. D. Phelps and B. G. Gegenbach. 1989. Restriction fragmenr length polymorphism in subclass of the 'Mandarin' soybean cytoplasm. Crop Sci. 29:1554-1559.

Grabau, E. A., W. H. Davis, N. D. Phelps and B. G. Gegenbach. 1992. Classification of soybean cultivars based on mitochondrial DNA restriction fragment length polymorphism. Crop Sci. 32:271-274.

Hanlon, R. and E. A. Grabeu. 1995. Cytoplasmic diversity in old domestic varieties of soybean using two mitochondrial makers. Crop Sci. 35:1148-1151.

Hirata, T., M. Kaneko, J. Abe, and Y. Shimamoto. 1994. Genetic structure of cultivated soybeans in Japan. Toward Enhanced and Sustainable Agricultural Productivity in the 2000's: Breeding Research and Biotechnology - Proceeding of the 7th International Congress of the SABRAO and WSAA. pp241-246.

Hirata, T., M. Kaneko, J. Abe, and Y. Shimamoto. 1996. Genetic differentiation between summer and autumn maturing cultivars of soybean (Glycine max (L.(Merrill) in Kyushu district of Japan. Euphytica 86. (in press)

Hymowitz, T. and N. Kaizuma. 1979. Dissemination of soybean (Glycine max): seed protein electrophoresis profiles among Japanese cultivars. Econ. Bot. 33:311-319.

Hymowitz, T. and N. Kaizuma. 1981. Soybean seed protein electrophoresis profiles from 15 Asian countries or region: Hypotheses on paths of dissemination of soybeans from China. Econ. Bot. 35:10-23.

Kishima, Y. 1988. Molecular and phylogenetic studies in the organelle genome variations among sugar beet (Beta Vulgaris L.) and its related species. (Doctoral dissertation), Hokkaido University. pp22-37. In Japanese.

Lee, D. J., C. A. Caha, J. E. Specht, and G. L. Graef. 1994. Analysis of cytoplasmic diversity in an outcrossing population of soybean. Crop Sci. 34:46-50.

Nagata, T. 1959. Studies on the differentiation of soybeans in the world, with special regard to that in southeast Asia: 2. Origin of culture and paths of dissemination of soybean, as considered by distributions of their summer vs. autumn soybean habit and plant habit. Proc. Crop Sci. Soc. of Jap. 28:79-84.

Schuster, W. ans A. Brennicke. 1987. Nucleotide sequence of the Onothera ATPase subunit6 gene. Nucl. Acids Res. 15:90-92.

Senda, M., T. Harada, T. Mikami, M. Sugiyama, and T. kinashita. 1991. Genomic organization and sequence analysis of the cytochrome oxidase subunit II gene from normal and male-sterile mitochondria in sugar beet. Curr Genet. 19:175-181.

Shimamoto, Y., A. Hasegawa, J. Abe, M. Ohara, and T. Mikami. 1992. Glycine soja germplasm in Japan: Isozyme and chloroplast DNA variation. Soybean Genet. Newsl. 19:73-77.

Toshiyuki HIRATA

Jun ABE

Yoshiya SHIMAMOTO

ATOMIC ENERGY AGRICULTURAL RESEARCH CENTRE
Tandojam, Pakistan

Impact of Grain Filling Period and Other Morphological Traits in Soybean

Abstract

Seventy six genotypes of diverse origin collected from different organizations within and outside Pakistan were planted in RCB design with two replications during summer 1994. Observations on various morphological characters like days to flower, days to mature, grain filling period, plant height, number of branches per plant, pod length, pods/plant, 100 grain weight and grain yield per plant were recorded for computing their correlation coefficients. Grain filling period along with other attributes e.g. plant height, no. of branches per plant, pods per plant and 100 grain weight showed positive and significant correlation with grain yield. It may be concluded that for selecting ideotypes with high grain yield potential in soybean, maximum selection pressure should be exerted on pods per plant, plant height, branches per plant and grain filling period.

Introduction

Among the newly introduced non traditional oilseed crops, soybean is one of the most promising oilseed crop which can play an important role to meet the edible oil requirements in which Pakistan is chronically deficient and is spending a huge amount of foreign exchange on its import. Selection for high seed filling period has been suggested as a mean of increasing yield in several crop species (Daynard et al. 1971, Carter and Poneleit 1973, Rasmusson et al. 1979. Jones et al. 1979, Reicosky 1982, Egli et al. 1984, Smith and Nelson 1986 a, b and Rodriguezde et al. 1991). For corn and soybean, a positive relationship has been found between length of seed filling period and yield (Daynard et al. 1971). Hanway and Weber 1971, Dunphy et al. 1979, and Reicosky et al. 1982, reported no correlation between the length of seed filling period and flowering date but suggested that the time between flowering and maturity could be used as an initial selection criterion for identifying lines with long seed fill. Nelson 1988, observed that selection for early flowering can have positive effect on seed yield by lengthening seed filling period.

Keeping all this information in view, present study was therefore, undertaken to asses the correlation coefficient between 10 different morphological attributes in 76 germ-plasm lines of soybean. It will help in isolating the most promising lines for using in our future breeding program.

Material and Methods

Seventy six diversified stable genotypes of soybean collected from different research organizations were evaluated in the southern

part of Pakistan at the experimental farm of Atomic Energy Agricultural Research Centre, Tandojam during summer 1994 (June-October). Single row of four meter length was planted in two replications in RCB design keeping inter and intra row spacing of 10 and 45 cm respectively. Flowering period was recorded when 50% plant population achieved flowering stage. The grain filling period (R_4 or R_5-R_7) was noted as described by Gay et al. (1980) and Dunphy et al. (1979). Maturity period was recorded when more than 90% of plant population was showing yellowing of the pods. At maturity, 10 randomly selected plants were taken and agronomic data regarding plant height, number of branches/plant, number of grains per pod, pods per plant, 100 grain weight, and grain yield per plant were recorded.

The data thus collected were subjected to analysis of variance and correlation coefficient values were worked out following the procedures of Steel and Torrie (1960) and Singh and Chaudhry (1979).

Results and Discussion

Days to flower showed highly significant positive correlation coefficient (r. 589) with days to mature, while it exhibited significant negative correlation with grain filling period (r-.233) and 100 grain weight (r-.247). It's correlation coefficient with grain yield was negative and non significant. Days to mature showed highly significant positive correlation (r.585) with grain filling period and significant and positive with pods per plant and significantly negative (r-.259) with 100 grain weight. Grain filling period exhibited significant positive correlation with pods per plant (r.288) and grain yield (r.300). Plant height showed highly significant positive correlation with number of branches per plant (r.316), pods per plant (r.359) and grain yield (r.445). Number of branches exhibited highly significant positive correlation with pose per plant (r.544) and grain yield (r.529). Pod length showed highly significant negative correlation with pods per plant (r-.447) and highly significant positive correlation with 100 grain weight (r.371). Seeds per pod showed non significant correlation with pods per plant, 100 grain weight and grain yield per plant. Pods per plant exhibited highly significant positive correlation with grain yield (r.701). 100 grain weight also showed significant and positive correlation with grain yield (r.299).

Over all grain filling period, plant height, number of branches per plant, pods per plant and 100 grain weight showed positive and significant correlation with grain yield. Pods per plant showed highest correlation with grain yield indicating that this character is the most important component of seed yield in soybean. Earlier, Chand et al. (1975), Katiyar et al. (1977), Rajput and Sarwar (1986) and Aslam et al.(1992) had also noted quite a large values of correlation coefficients and concluded that while selecting for high yield in soybean, main emphasis should be placed on pods per plant.

Grain filling period showed significant positive association with grain yield. Williams et al. (1979), Boote (1981), Nelson (1988), and Blain and Hume (1990), also found positive association of seed yield with larger seed filling period, while Dunphy et al. (1970) observed that high yield was associated with both late flowering and late

maturity. Considering the results of present study it is suggested that for selecting ideotype with high seed yield potential in soybean, maximum selection pressure should be exerted on pods per plant followed by plant height, branches per plant, and grain filling period.

References

Aslam, M., N. A. Khan, S. M. Mirza, and A. Rehman Khan. 1992. Correlation and path coefficient analysis for yield components in soybean. Pakistan. J. Agric. Res. 13(1):20-25.

Blain, Mc. and D. J. Hume. 1980. Physiological studies of higher yield in new early maturity soybean cultivars. Can. J. Plant Sci. 60: 1315-1326.

Boote, K. J. 1981. Response of soybean in different maturity groups to March planting in Southern U. S. A. Agron. J. 73:854-859.

Carter, M. W., and C. G. Poneleit. 1973. Black layer maturity and filling period variation among inbred lines of Corn (*Zea mays* L.) Crop Sci. 13:436-439.

Chand, H. E. S, Srivastava and K. B. Trehan. 1975. Estimates of genetic parameters, correlation coefficient and path coefficient analysis in gram (*Cicer arietinum* L.) Madras Agri. J. 62(4): 178-181. (Pl. Breeding Abstr. 47(1):896, 1977.)

Daynard T. B., J. W. Tanner, and W. G. Duncan. 1971. Duration of the grain filling period and its relation to grain yield in corn. Crop Sci. 11:45-48.

Dunphy, E. J. Hanway, and D. E. Green. 1979. Soybean yields in relation to days between specific developmental stages. Agro. J. 71(6):917-920.

Egli, D. B., J. H. Orf, and T. W. Pfieffer. 1984. Genotypic variation for duration of seed fill in soybean. Crop Sci. 55(3):587-592.

Gay, S., D. B. Egli, and D. A. Reicosky. 1980. Physiological aspects of yield improvement in Soybean. Agron J. 72:387-391.

Hanway, J. J., and C. R. Weber. 1971. Dry matter accumulation in eight soybean (*Glycine max* (L) Merrill) varieties. Agron. J. 63: 227-230.

Jones, D. B., M. L. Petroson, and S. Geng. 1979. Association between grain filling rate and duration and yield in Rice. Crop Sci. 19(1):641-644.

Katiyar, R. P., J. Prasad, A. B. Singh, and K. Raw. 1977. Association analysis of grain yield and its components in segregation populations of chickpea. Indian J. Agric. Sci. 47(7): 325-327. (Field Crop Abstr. 32(1):319, 1970).

Nelson, R. L. 1988. Response to selection for time of flowering in soybean. Crop Sci. 28(4):623-625.

Rajput, M. A., G. Sarwar, and K. H. Takir. 1986. Path coefficient analysis of developmental and yield components in soybean. Soybean Genetic Newsletter. 13:87-91.

Rasmusson, D. C., D. I. Melcan, and T. L. Tew. 1979. Vegetative and grain filling periods of growth in barley. Crop Sci. 19:5-9.

Reicosky, D. A., J. H. Orf, and C. Poneleit. 1982. Soybean germplasm evaluation for length of the seed filling period. Crop Sci. 22:319-322.

Rodriguezde, C. S., D. E. Green, C. S. Chang, and R. M. Shibles. 1991. Developmental period in soybean photo-period sensitive and insensitive crosses evaluated at diverse latitudes. Crop Sci. 31(1):8-13.

Singh, R. K., and B. D. Chaudhry. 1979. Biometerical methods in quantitative genetic analysis. Kalyani Publishers Ludhiana New Dehli, India.

Smith, J. R., and R. L. Nelson. 1986a. Selection for seed filling period in soybean. Crop Sci. 26:466-469.

Smith, J. R., and R. L. Nelson. 1986b. The relationship between seed filling period and yield among soybean breeding lines. Crop Sci. 26:469-472.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw Hill Book Inc., New York.

Williams, W. A., C. Q. Qualset, and S. Geng. 1979. Ridge regression for extracting soybean yield factors. Crop Sci. 19:869-873.

G. Sarwar

M. A. Rajput

K. A. Siddiqui

N. A. Shaikh

M. A. Siddiqui

Table 1: Correlation coefficient among different morphological characters of soybean during 1994.

Characters	Days to flower	Days to mature	Grain filling period	Plant height	No. of Branches /Plant	Pod length	Seeds/ pod	Pods/ plant	100 grain weight	Grain yield/plant
Days to flower	**									
Days to mature	0.589	**								
Grain filling period	-0.233*	0.585								
Plant height	0.113	0.115	0.075							
No. of Branches/plant	0.063	0.167	0.225	0.316**						
Pod length	-0.113	-0.206	-0.159	0.179	-0.155					
Seeds/pod	-0.011	0.108	0.101	0.280*	0.102	0.269*				
Pods/plant	0.191	0.281*	0.288*	0.359**	0.544**	-0.447**	0.013			
100 Grain Weight	-0.247*	-0.259*	-0.038	-0.114	-0.059	0.371**	-0.013	-0.165		
Grain yield/plant	-0.055	0.086	0.300*	0.445**	0.529	-0.013	0.155	0.701**	0.299*	

AGRICULTURAL UNIVERSITY
Department of Genetics and Plant Breeding
ul. Wojska Polskiego 71c
60-625 Poznan, Poland

Regeneration of Soybean plants from embryonic axes

Several parts from immature embryos, immature and mature seeds, and seedlings were used as explants for regeneration of soybean plants in in vitro culture. Cotyledons of immature embryos were usually used for regeneration by somatic embryogenesis (Lazzeri et al., 1987; Komatsuda and Ohyama, 1988; Christou and Ning-Sun Yang. 1989). The cotyledonery nodes were common explants for regeneration by shoots formation (Wright et al., 1986). However, Mc Cabe et al, (1988) got a stable transformed soybean plants using embryonic axes as explants. Christou et al. (1990) described the method for production of transgenic plants and established (Christou, 1994) that embryonic axes from mature and immature seeds were the best explant source for transformation by particle acceleration by electric discharge. Following this way, at the first step we checked ability of Polish genotypes to regeneration from embryonic axes.

Materials and Methods

Polish soybean cultivars Nawiko and Aldana, and six lines derived from *Glycine max* x *G. soja* crosses were examined. All genotypes were grown in the greenhouse in 1995. Four to five weeks after flowering pods were collected and sterilized in 70% ethanol for 1 min and in 6% calcium hypochlorite for 15 min. Immature seeds at 7-8 mm size were removed from pods and embryonic axes were excised. Explants were plated on four induction media. The following media were used: K1 - MS (Murashige and Skoog, 1962) basal medium with 1.38 g l^{-1} L-proline, 3.0 mg l^{-1} BA (N-6-benzyadenine), 0.4 mg l^{-1} NAA (α - naphtalene acetic acid); K2 - MS macronutrient salts, four times the MS level of micronutrient salts, B5 (Gamborg et al., 1968) vitamins with 3.0 mg l^{-1} BA and 0.4 mg l^{-1} NAA; K3 - MS macronutrient salts, four times the MS level of micronutrient salts, B5 vitamins with 6.0 mg l^{-1} BA and 0.4 mg l^{-1} NAA; K4 - MS macronutrient salts, four times the MS level of micronutrient salts, B5 vitamins with 3.0 mg l^{-1} BA. All the media contained 30 g l^{-1} of sucrose and 7 g l^{-1} of agar. From 16 to 20 explants were plated from each combination. After two weeks of induction in darknes at 22°C, explants were transferred on fresh medium based on MS salts with 0.38 mg l^{-1} BAP and 0.0372 mg l^{-1} NAA and taken to the culture room under a 16-h photoperiod. After 4, 8 and 12 weeks of culture, shoots with 2 or 3 leaves were cut off and transferred on B5 medium modified by 0.02 mg l^{-1} NAA. Plantlets with well developed roots were potted in soil (50% soil, 50% perlite) and transferred to the greenhouse.

Results and Discussion

During two weeks in the darkness, embryonic axes have lost their green colour. Transferred to the light explants quickly became green and after one week first shoots appeared. Regeneration from the area of primary and axillary meristems was observed. Small explants did not produce shoots, thus number of shoots produced by individual explant varied from 0 to 12. The oldest explants produced more shoots than the younger ones. These observations agree with Christou et al. (1990) who reported that the best age of explants of embryonic axes was 30-40 days post-flowering.

All genotypes produced shoots and showed similar responses of regeneration (Table 1). Only two lines (716 and 420) produced a slightly higher number of shoots. The highest average number of shoots per explant (4.7) were observed in line 716 on K2 medium. Percent of shoots with well developed roots depended on genotype (Table 1), but only shoots longer than 1 cm were transferred on rooting medium. Comparetively low percent of rooted shoots probably resulted from medium and too small shoots transferred on the medium. Generally, more plants from lines derived from *Glycine max* x *G. soja* crosses were regenerated (Table 1). K2 medium developed by Mc Cabe et al. (1988) was the best for shoots formation of all Polish genotypes (Table 2).

Jerzy Nawracala
Grzegorz Konieczny
Mariusz Kazmierczak

Table 1. Genotype responses in shoot formation from embryonic axes cultured on four induction media.

Genotype	Shoots per explant	Range dependent on medium	Percent of rooted shoots	Number of developed plants
Aldana	2.4	1.6 - 3.2	46.6	34
Nawiko	2.6	1.7 - 3.9	61.7	71
PGR 294	2.4	1.5 - 3.3	61.7	91
420	3.4	2.9 - 3.9	75.9	142
426	2.6	1.3 - 3.4	78.1	114
709	2.7	1.9 - 3.4	67.7	111
716	3.6	2.2 - 4.7	52.9	90
1017	2.4	1.8 - 3.0	77.5	55

Table 2. The effect of medium on shoot regeneration from embryonic axes of eight soybean genotypes.

Medium	Shoots per explant	Range dependent on genotype	Percent of rooted shoots	Number of developed plants
K 1	2.9	1.6 - 3.9	68.3	190
K 2	3.4	2.4 - 4.7	64.0	240
K 3	2.8	1.8 - 3.6	66.0	169
K 4	1.9	1.3 - 2.5	66.1	109

References

Christou, P., D. E. Mc Cabe, B. J. Martinell, and W. F. Swain. 1990. Soybean genetic engineering - commercial production of transgenic plants. TIBTECH 8:145-151.

Christou, P. 1994. Genetic engineering of crop legumes and cereals: current status and recent advances. Agro-Food - dustry Hi - Tech March/April 17-27.

Christou, P. and Ning-Sun Yang. 1989. Developmental aspects of soybean (*Glycine max*) somatic embryogenesis. Ann. of Bot. 64:225-234.

Gamborg, O. L., R. A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Reasearch 50:151-158.

Lazzeri, P. A., D. F. Hildebrand, and G. B. Collins. 1987. Soybean somatic embryogenesis: effects of hormones and culture manipulation. Plant Tiss. Organ Cult. 10:197-208.

Komatsuda, T. and K. Ohyama . 1988. Genotypes of high competence for somatic embryogenesis and plant regeneration in soybean *Glycine max*. Teor. Appl. Genet. 75:695-700.

Mc Cabe, D. E., W. F: Swain, B. J. Martinell, and Christou, P. 1988. Stable transformation of soybean (*Glycine max*) by particle acceleration. Bio Technology 6:923-926.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15:473-497.

Wright, M. S., S. M. Koehler, M. A. Hinchee, and M. G. Carnes. 1986. Plant regeneration by organogenesis in *Glycine max*. Plant Cell Rep. 5:150-154.

KASETSART UNIVERSITY
Faculty of Science
Department of Applied Radiation and Isotopes
Bangkok 10900
Thailand

Observation on Unstable Genes of Flower and Seed Coat Colors in Soybeans Derived from a "Wakashima" Mutant.

White - flowered "Wakashima" mutants were obtained from purple - flowered "Wakashima" cultivar in 1974 (Singburaudom, 1977).

In 1978, seeds of white - flowered "Wakashima" mutants were irradiated. After planting treated seeds the purple - flowered plants were obtained. Approximately 7 per cent of back mutation could be recorded (Smutkupt, Wongpiyasatid and Lamseejan, 1985).

Later, various mutants including "Wakashima" mutant derived lines were established in 1981 (Vipasrinimit, 1979; Noree, 1981).

In 1984, in order to observe mutations of flower and seed coat colors, 15 seeds of single plants were grown in 3 pots, 5 seeds in each pot. At flowering time, the flower color of each plant was recorded. Later, each plant was singly harvested and threshed; its seed coat color was recorded.

It was interesting to note that a single white - flowered plant derived from 5 brown seeds of a plant in 81-1-145 line planted on August 31, 1984 yielded 48 grams of yellow seeds. Twenty seeds of them were immediately planted on February 1, 1985 in 4 earth pots with 5 seeds in each pot.

Segregations of flower and seed coat colors of plants survived in each pot were observed as follows :

In the first pot, 3 purple - flowered plants yielded yellow seeds. In the second pot, 2 plants were obtained. The first plant had purple flowers and gave black seeds, the other plant had white flowers and yielded yellow seeds.

Two plants survived in the third plot. Both plants had white flowers and gave yellow seeds.

In the fourth pot, 5 plants survived. One plant had purple flowers and yielded black seeds. The other four plants had purple flowers and yielded yellow seeds.

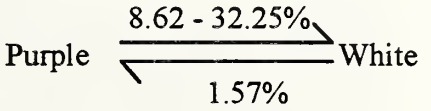
Later in 1986, the plants derived from those observed plants were divided into three groups, as I-7, I-66, and I-28 for further observation. Pot experiments in larger plant population using seeds derived from a single plant and planting 5 seeds per pot were carried out. The results are shown in Table 1.

Table 1. Segregation of flower and seed coat colors in soybean 81-1-145 derived lines.

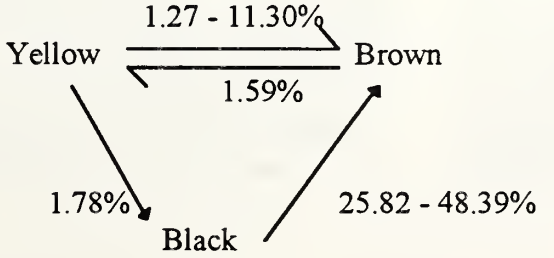
Parents' characteristics		Number of plant progenies survived	Flower and seed coat color of plant progenies					
Colors of flower and seed coat	No. of seeds planted		White flower			Purple flower		
			seed coat color			seed coat color		
			yellow	black	brown	yellow	black	brown
I -7								
White yellow	90	79	78 (98.73%)	-	1 (1.27%)	-	-	-
White black	45	31	-	16 (51.61%)	15 (48.39%)	-	-	-
Purple yellow	245	225	47 (20.88%)	-	2 (0.88%)	160 (71.11%)	4 (1.78%)	12 (5.33%)
Purple black	80	58	-	5 (8.62%)	5 (8.62%)	-	33 (56.89%)	15 (25.82%)
Purple brown	100	90	-	-	13 (14.45%)	-	-	77 (88.55%)
I - 66								
White yellow	77	64	63 (98.43%)	-	-	1 (1.57%)	-	-
Purple yellow	65	62	20 (32.25%)	1 (1.61%)	-	41 (66.11%)	-	-
I - 28								
White yellow	70	62	55 (88.7%)	-	7 (11.30%)	-	-	-
White brown	70	63	1 (1.59%)	-	62 (98.41%)	-	-	-

Mutations of flower color and seed coat characteristics of soybean lines derived from a "Wakashima" mutant line 81-1-145 were observed and mutation rate of single and double mutations were recorded and can be summarized as follows:

1. Flower colors



2. Seed coat colors



3. Double mutations

3.1 Purple flower/yellow seed $\xrightarrow{0.88\%}$ White flower/brown seed

3.2 Purple flower/black seed $\xrightarrow{8.62\%}$ White flower/brown seed

3.3 Purple flower/yellow seed $\xrightarrow{1.61\%}$ White flower/black seed

The back mutations of seed coat colors from brown to black (0.31%) and black to yellow (1.11%) were observed in later experiments carried out in December 1987 - April 1988. Lines derived from 81-1-145 are maintained for genetic studies.

Sumin Smutkupt

References

1. Noree, P. 1981. Selection of M3 soybean lines for rust resistance. M.S. Thesis Kasetsart University (in Thai with English summary).

2. Singburaudom, N. 1977. Gamma irradiation induced mutation in the soybean, Glycine max (L.) Merr. M.S. Thesis Kasetsart University (in Thai with English summary).

3. Smutkupt, Wongpiyasatid and Lamseejan, 1985. Observation on back mutation of white - flowered "Wakashima" mutants. Soybean Genetics Newsletter 12:67-69.

4. Vipasrinimit, S. 1979. Selection of rust resistance in M3 soybean lines. M.S. Thesis. Kasetsart University (in Thai with English summary).

IOWA STATE UNIVERSITY
USDA/ARS Field Crops Research
Department of Agronomy
Ames, IA 50011

A Chimeric Soybean Plant

The Chimaera of Greek mythology was a 'fire-breathing monster, the foreparts of whose body were those of a lion, the middle parts those of a goat, and hinder parts those of a dragon.' (Neilson-Jones, 1969). The occurrence of plant chimeras in nature is a common phenomenon. Chimeras are a direct result of the segregation of normal and mutant organelles into genotypically and phenotypically distinct vegetative sectors. Progeny of chimeric plants manifest a direct relationship between sector phenotypes and nuclear or cytoplasmic genotypes.

Five of the eight cytoplasmically - inherited chlorophyll mutants in soybean are direct descendents of chimeric plants (Palmer and Mascia, 1980; Shoemaker et al., 1985; Cianzio and Palmer, 1992). We report the occurrence of a chimeric plant (Table 1) and genetic studies of the yellow foliage progeny of the original chimeric plant (Table 2).

The original cross was A88-406 (female) x A88-362 (male). Entry 406 was T323, Mdh1-n (Ames 2) y20 (Ames 2), a malate dehydrogenase 1 null, chlorophyll-deficient mutant, which was isolated in a gene tagging experiment with w4-m mutable allele (Palmer et al., 1989; Hedges and Palmer, 1992). Entry 363 was Clark-k_2 (L67-3483) a tan saddle mutant found in X-ray radiated Clark. The objective of the cross was to obtain a genetic recombination (repulsion phase) to produce Mdh1-n (Ames 2) y20 (Ames 2) k_2. The F_2 generation segregated as expected for plant color, malate dehydrogenase, and tan saddle. A number of F_2 plants were threshed individually and planted as progeny rows. In the F_3 generation in entry A94-B19, segregation was observed for green and yellow plants, and one chimeric plant was identified which had tan saddle seed coat. Progeny of this chimeric plant, A95-B66, were purple flower, uniformly yellow foliage and had tan saddle seed coat. Reciprocal cross pollinations were made with cultivar Evans (white flower). The F_1 plants were grown in Puerto Rico at the Isabela Substation. The F_2 seedlings were evaluated in the sandbench in the glasshouse in Ames, Iowa. All F_2 progenies were of hybrid origin as shown by segregation of hypocotyl color (Table 2). All F_1 and F_2 plants were green foliage when Evans was the female parent and all F_1 and F_2 plants were yellow foliage when A95-B66 was the female parent.

Three seed from each of six plants of A95-B66 (F_5 seed) were tested electrophoretically for malate dehydrogenase (Cardy and Beversdorf 1984). All seed had malate dehydrogenase (Mdh1). The presence of Mdh1 and the absence of yellow plants (y20) in the F_2 of A95-1444 x B66 (Table 2),

indicate that the A95-B66 yellow plants are not the result of a recombinational event involving the k_2 Mdh1 y20 loci.

Summary:

We believe that A95-B66 yellow plants are the result of a cytoplasmically inherited trait. Additional data are needed to confirm our hypothesis. The occurrence of all yellow plants (total 168) among the progeny of the original chimeric plant (A94-B19-1) is not immediately apparent.

References

Cardy, B. J. and W. D. Beversdorf. 1984. A procedure for the starch gel electrophoretic detection of isozymes in soybean [*Glycine max* (L.) Merr.]. Dept. Crop Sci. Tech. Bull. 119/8401. University of Guelph.

Cianzio, S. R. and R. G. Palmer. 1992. Genetics of five cytoplasmically inherited yellow foliar mutants in soybean. J. Hered. 83:70-73.

Hedges, B. R. and R. G. Palmer. 1992. Inheritance of malate dehydrogenase nulls in soybean. Biochem. Genet. 30:491-592.

Neilson-Jones, W. 1969. Plant chimeras. Methuer and Co. Ltd. London.

Palmer, R. G. and P. N. Mascia. 1980. Genetics and ultrastructure of a cytoplasmically - inherited yellow mutant in soybeans. Genetics 95:985-1000.

Palmer, R. G., B. R. Hedges, R. S. Benavente, and R. W. Groose. 1989. w4-mutable line in soybean. Dev. Genet. 10:543-551.

Shoemaker, R. C., A. M. Cody, and R. G. Palmer. 1985. Characterization of a cytoplasmically inherited yellow foliar mutants (cyt-Y3) in soybean. Theor. Appl. Genet. 69:279-284.

R. G. Palmer - USDA
Xianfeng Chen

Table 1. Description of chimeric soybean plant from a cross of T 323 x L 67-3483 [a].

Plot no.	Generation	Phenotype
A88-406	Parent	Yellow
A88-362	Parent	Green
A89-503-1	F_1	Green
A93-B283-1	F_2	Segregating plant color, malate dehydrogenase, tan saddle seed coat
A94-B19-1	F_3	Segregating plant color, malate dehydrogenase, tan saddle seed coat; selected 1 chimeric plant
A95-B66	F_4	Yellow (168 plants), all tan saddle seed coat

[a] T323 is Mdh1-n (Ames 2) y20 (Ames 2) and L67-3483 is Clark-k_2.

Table 2. Reciprocal crosses of yellow plants (A95-B66) x cultivar Evans (A95-1444); F_1 and F_2 data.

	F1 plants		F2 plants	F2 plants, hypocotyl color		
Cross	No.	Phenotype	Phenotype	Purple	Green	χ^2 (3:1)
A95-1444 x B66	8	Green	Green	387	126	0.05
A95-B66 x 1444	5	Yellow	Yellow	248	86	0.10

IOWA STATE UNIVERSITY
USDA/ARS Field Crops Research
Department of Agronomy
Ames, IA 50011

Construction and Size Characterization of a Bacterial Artificial Chromosome (BAC) Library from Soybean

We have constructed a BAC library suitable for map-based cloning and physical mapping in soybean using DNA from the soybean cultivar Williams 82. This library represents 4.0 to 5.0 genome equivalents (40,000 clones) with an average insert size of 150 kb. This communication describes how we made the library and characterized it based on insert size.

Megabase DNA Preparation

Megabase DNA was prepared in agarose microbeads using a combination of the methods described by Woo et al. (1994), Liu and Whittier (1994), and Koob and Szybalski (1992). A nuclei enriched fraction was prepared by grinding young Williams 82 leaves (either primary leaves or first trifoliates before they were completely expanded) to a very fine powder in liquid N_2. The leaves were usually cut and kept wrapped in the dark at 4C for 48 hours before grinding. 20-26 g of leaf powder were added to 200 mL of ice-cold isolation buffer stirring on ice [10 mM Tris, 10 mM EDTA, 60 mM NaCl, 4 mM spermidine, 1 mM spermine, 0.5 M sucrose, 600 uL NaOH/400 mL buffer; 400 uL of beta mercaptoethanol (BME) was added just before use]. After 10 min, the mixture was filtered through 2 layers of Miracloth into a 250 mL flask. Eight mL of 10% Triton X-100 were added and the flask gently swirled for 2 min. The prep was divided among 50 mL centrifuge tubes and centrifuged for 15 min at 2500 rpm (approx 1400xg) to pellet the nuclei. The supernatent was discarded, the pellets resuspended in small volumes of isolation buffer, combined into one 50 mL tube and the volume brought to 45 mL with isolation buffer. The nuclei prep was centrifuged 10 min at 2500 rpm and the pellets resuspended and washed 2 more times in isolation buffer. The last resuspension was made with 1 mL of isolation buffer without BME. The nuclei prep was warmed to approximately 43C before adding an equal volume of 1.2% InCert agarose (43C, dissolved in isolation buffer without BME). The nuclei/agarose mix was quickly added to mineral oil in a 50 mL flask (both at 43C; oil volume equal to the volume of the nuclei plus agarose), vortexed at full speed for 12-15 sec, then swirled rapidly in a 5 M NaCl ice bath for about 90 sec to harden the microbeads. A volume of ice-cold isolation buffer without BME equal to the volume of oil was added, the flask mixed vigorously, and the contents poured into a 50 mL centrifuge tube. The microbeads were centrifuged for 15 min at 1800 rpm (approx 750xg), resuspended in 40 mL lysis buffer (0.1% sarkosyl, 0.2 mg/mL proteinase K in 0.5 M EDTA pH 9.4) and incubated at 48C with very gentle swirling for approximately 48 hr with one change of

lysis buffer after 24 hr. The microbeads were pelleted (5 min 1200 rpm, approx 350xg), resuspended in 0.5 M EDTA pH 9.4 and incubated at 48C for 1 hour. The microbeads were again pelleted, resuspended in 50 mM EDTA pH 8.0 and put on ice (or refrigerated) for at least an hour before beginning washes to remove the detergent and proteinase K. Before use, the microbeads were washed for 1x 30 min in TE (10 mM Tris pH 8.0, 1 mM EDTA) 1 mM phenylmethylsulfonyl fluoride (PMSF), followed by 2x 30 min washes in TE 0.1mM PMSF. The micobeads were then washed in TE 3x for 60 min each and stored at 4C until use.

pBeloBAC11 Vector Preparation

pBeloBAC11, a single copy 7.3 kb F-based plasmid developed by Dr. Hiroaki Shizuya (Shizuya et al 1992) was obtained in DH10B *E. coli* cells with the kind permission of Dr. Shizuya from Dr. Rod Wing at Texas A and M University. A single colony was used to innoculate a 10 mL overnight culture which was used to seed 6 cultures of approximately 670 mL each which were grown at 37C for 20 hr. Plasmid DNA was isolated using the standard alkaline lysis maxiprep method described in Sambrook et al. (1989) followed by a precipitation with equal volumes of 5 M LiCl to remove RNA and precipitation of the DNA enriched nucleic acid fraction with isopropanol. Plasmid was purified by CsCl/EtBr gradient centrifugation (50000 rpm TV-865 rotor, Sorvall Ultracentrifuge). EtBr was removed from the plasmid band by extraction with TE saturated butanol. The DNA was then diluted with 3 volumes of TE and precipitated with 2 volumes of ethanol. The pellet was washed with 70% ethanol and the plasmid DNA resuspended in TE aliquoted and stored at -20C.

Vector was prepared for use in library construction essentially as described by Woo et al. (1994). An aliquot containing 10 ug of DNA was digested with HindIII for 3-4 hr in TA buffer (Epicentre Technologies; 33 mM Tris-acetate pH 7.8, 66 mM K-acetate, 10 MM Mg-acetate, 0.5 mM DTT, 100ug/mL BSA). A second aliquot of HindIII was added after 2 hr. After adding $CaCl_2$ to a final concentration of 5 mM, the mixture was equilibrated at 30C for 5 min and then 1 U/ug DNA of HK phosphatase (Epicentre Technologies) was added. After a 1 hr incubation at 30C, the HK phosphatase was inactivated by incubating at 65C for 30 min. The vector prep was extracted once with an equal volume of phenol/chloroform and once with an equal volume of chloroform before precipitating the DNA with Na-acetate and ethanol. The vector DNA was resuspended in TE, aliquoted, and stored at -20C.

Library Construction

80 uL of microbeads were aliquoted and used for partial digests with HindIII. A 5 min digest at 37C with 3 or 5 U of HindIII was typically used. The microbeads were incubated on ice in 0.5mL digestion buffer (10 mM Tris pH 7.5, 50 mM NaCl, 1mM DTT, 4 mM spermidine, 1mg/mL BSA). After 30 min, excess buffer was removed, a second 0.5 mL aliquot of digestion buffer added and the microbeads incubated for an additional 30 min. Excess digestion buffer was removed (approximately 100 uL remained), enzyme added and the beads incubated for 20-30 min on ice before adding $MgCl_2$ (final conc 6 mM) and incubating at 37C. The digestion was terminated by adding 0.1 volume of 0.5 M EDTA pH 8.0 and incubating the reactions on ice for 10 min. The partial digests were loaded onto a 1% low melt agarose gel and electrophoresed at 190V in a BioRad CHEF apparatus for 22

hours using a 90 sec switch time in TAE buffer. Upon completion of the electrophoresis the appropriate segments (usually the 300-600 kB range as judged by a lambda concatamer ladder included on the gel) were cut from the gel and either the agarose digested (Gelase, Epicentre Technologies) and the DNA used for ligations or the segments were inserted into a second 1% low melt agarose gel and electrophoresed for 10 hours at 140V with a 5 sec switch time. The compressed DNA band at 100 kB and above was cut from the second size selection gel and digested with Gelase. The digested agarose DNA preps were used for 100 uL ligations with 10-20 ng vector, 1 mM ATP and 2U ligase (Epicentre T4 DNA Ligase). The ligations were incubated at 16C for 8-14 hours, heated to 65C for 10 min and used for electroporation at 1.9kV in a BioRad Gene Pulser. 1 uL of the ligation mixture was electroporated into approximately 27 uL of electromax competent DH10B cells (Gibco/BRL). Typically, the ligation mixture needed to be diluted to allow for maximum efficiency of transformation. An optimal dilution was determined empirically. Electroporated cells were diluted immediately with 1 mL of SOC (2% Bacto-tryptome, 0.5% Bacto-yeast, 10 mM NaCl, 2.5 mM KCl, 5 mM $MgCl_2$, 5 mM $MgSO_4$, 20 mM glucose, pH 7.0), transferred to microfuge tubes and incubated at 37C for 40 min before plating on selective medium (LBplates with 12.5 ug/ml chloramphenicol, 0.55 mM IPTG and 80 ug/mL X-gal) The plates were incubated at 37C for approximately 24 hours and then refrigerated at 4C for 2 days or more before picking the white recombinant colonies for our library. Clones were stored in freezing broth (36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM Na citrate, 0.4 mM MgSO4, 6.8 mM $(NH_4)_2SO_4$, 4.4% v/v glycerol in LB) in 384 well microtiter dishes in a -80 freezer.

Library Characterization

To obtain an estimate of average insert size, random BACs were picked from several different transformation plates from each ligation and used to grow 5mL LB chloramphenicol (12.5 ug/mL) overnight cultures. Standard alkaline lysis plasmid minipreps (Sambrook et al. 1989) were done using 1.5 mL of the cultures and 3-5 uL of the preps digested with NotI (1.5 hr digest using 10U NotI). There are 2 NotI sites in peloBAC11 just outside of the multicloning site. Because NotI is an 8 base cutter, digestion typically generates vector plus a complete insert. Digests were electrophoresed in a 1% agarose TBE gel at 190V for 22 hours on a BioRad CHEF apparatus using a 8-30 sec linear ramp time. As shown in Table 1, the average insert size of our library clones using several microbead preps and numerous partial digests and ligations is 150 kb.

We have generated between 4 and 5 genome equivalents of clones for the library. We hope to have the library available for public use most probably by PCR screening of pooled sets of BACs by the end of the summer 1996.

Table 1. Average Insert Size of Soybean BAC Library

BAC prep	Number of Colonies Sampled	size range,kb	average size,kb
09.17	15	60-220	130
09.19	22	40-235	138
09.25	23	70-370	160
09.28	25	90-280	162
10.02	15	55-190	129
10.10	26	50-300	162
12.11	17	140-230	155
12.15	27	90-235	158
01.03	24	70-220	154
01.03	30	115-230	145
		average size	151

References

Koob, M. and W. Szybalski. 1992. Preparing and using agarose microbeads. Methods in Enzymology 216: 13-20.

Liu, Y. and R. Whittier. 1994. Rapid preparation of megabase plant DNA from nuclei in agarose plugs and microbeads. Nucleic Acids Res. 22:21168-2169.

Sambrook, J., E. Fritsch, and T. Maniatis. 1989. Molecular Cloning. A Laboratory Manual, 2nd Edition. Cold Spring Harbor Laboratory Press, New York.

Shizuya, H., B. Birren U-J. Kim, V. Mancino, T. Slepak, Y. Tachiri, and M. Simon. 1992. Cloning and stable maintenance of 300 - kilobase - pair fragments of human DNA in Escherichia coli using a F-factor-based vector. Proc. Natl. Acad. Sci. (USA) 89: 8794-8797.

Woo, S., J. Jiang, B. Gill, A. Paterson, and R. Wing. 1994. Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. Nucleic Acids Res. 22: 4922-4931.

Laura Fredrick Marek

Randy C. Shoemaker

USDA/ARS FIELD CROPS RESEARCH
IOWA STATE UNIVERSITY
Department of Agronomy
Ames, IA 50011

Inheritance of an Endopeptidase Null Mutant

Introduction:

Enzyme markers have been used for soybean genetic studies for a long time. The detection of isozymes as markers was used by Hunter and Markert (1957). A new variant, endopeptidase null (*Enp-null*), was found in USDA PI 567365 by using electrophoresis. The purposes of this study were to determine the inheritance of the endopeptidase null variant and to determine linkage relationships between endopeptidase null and other markers in soybeans.

Materials And Methods:

USDA PI 567365 (Qing Da Dou) was from Ningxia, China. Crosses of BSR101 (Tachibana et al. 1987) and PI 567365 and Minsoy (PI 27890) and PI 567365 (Table 1) were done in 1993 at Bruner Farm, near Ames, Iowa.

The F_2 seeds of these two crosses were used to identify isozyme genotypes and morphological traits. Isozyme electrophoresis was adapted from Rennie, et al. (1989) and Wendel and Weeden (1989).

A piece of cotyledon was sampled from each two-day-old F_2 seedling and was used for electrophoresis, and seedling roots were checked under the UV light to determine whether their roots were fluorescent or nonfluorescent. Identity of the seedlings was maintained and they were transplanted to the sandbench in greenhouse. The hypocotyl color and the pubescence tip of each seedling were recorded after about two weeks.

The data were analyzed by using the computer program LINKAGE-1, 1989 version (Suiter et al., 1983) to obtain recombination frequencies.

Results and Discussion:

Inheritance of endopeptidase null allele:

The data indicated that endopeptidase null was inherited as a single recessive nuclear gene. The segregation ratio from both crosses fit a 3:1 ratio.

Linkage determination:

The traits used in the two genetic linkage studies segregated in the expected ratio (Table 3). The data from Table 4 suggested that *Enp-null* had no linkage relationship with *Aco4*, Idh_1 , Fr_1, W_1, and *Pb* loci. At the same time, we also checked the gene pairs of *Aco4* -- W_1, *Aco4* -- *Pb*, *Pb* -- W_1, Idh_1 -- Fr_1, Fr_1 -- W_1, and Idh_1 -- W_1. No linkage relationships among these loci were detected (Table 5).

Summary:

The data from the two crosses were homogenous. Segregation for endopeptidase indicated that *Enp-null* was a single recessive Mendelian trait. No linkage relationships were found for five linkage tests with endopeptidase null. The linkage relationships between the other six loci indicated that these traits were not linked. These results were in same agreement with Griffin and Palmer (1987) who found that *Enp--Aco4*, Idh_1 *--Enp*, and *Enp--*W_1 were not linked. Therefore the new observations from this research were that *Enp--* Fr_1 and *Enp -- Pb* were not linked.

Wenying Liao
Xianfeng Chen
Reid G. Palmer, USDA-ARS

Table 1. Parents genotypes

	Aco4	*Enp*	*Idh1*	Root fluorescence	Hypocotyl color	Pubescence tip
PI 567365	*b/b*	*null*	*b/b*	Fr_1/Fr_1	w_1 / w_1	*Pb/Pb*
BSR101	*a/a*	*a/a*	*b/b*	Fr_1/Fr_1	W_1 / W_1	*pb/pb*
Minsoy	*b/b*	*a/a*	*a/a*	fr_1/fr_1	W_1 / W_1	*Pb/Pb*

Table 2. Inheritance of endopeptidase null (F_2 data)

	Enp-a	*Enp-null*	N	$X^2_{(3:1)}$	P
BSR101 X PI 567365	233	81	314	0.11	0.76
Minsoy X PI 567365	194	68	262	0.13	0.73

Table 3. Segregation at loci involved in inheritance and linkage studies (F_2 data)

Locus	Genotypes	Expected ratio	Observed ratio	N	$X^2_{(3:1)}$ or $X^2_{(1:2:1)}$	df	P
Aco4[a]	aa : ab : bb	1:2:1	73:157:76	306	0.27	2	0.88
Pb[a]	Pb_: pbpb	3:1	155:66	221	2.77	1	0.097
W_1[a]	W_1_ : w_1 w_1	3:1	166:55	221	0.0049	1	0.95
Idh_1[b]	a_: bb	3:1	187:73	260	1.33	1	0.25
Fr_1[b]	Fr_1_ : fr_1fr_1	3:1	183:60	243	0.01	1	0.93
W_1[b]	W_1_ : w_1 w_1	3:1	136: 44	180	0.03	1	0.88

* a: BSR101 X PI 567365
* b: Minsoy X PI 567365

Table 4. Linkage determination of the endopeptidase null mutant with other genetic markers (F_2 data)

Cross	Locus tested	N	Rec	+/- SE
BSR101 X PI 567365				
	Aco4	306	0.488	0.035
	Pb	222	0.474	0.052
	W_1	222	0.423	0.055
Minsoy X PI 567365				
	Idh_1	259	0.474	0.048
	Fr_1	242	0.490	0.049
	W_1	179	0.496	0.056

Table 5. Linkage determination of other genetic marker in the two endopeptidase null mutant crosses (F_2 Data).

Locus 1 – Locus 2	N	Rec	+/- SE
Aco4 -- W_1	222	0.484	0.035
Aco4 -- *Pb*	221	0.498	0.052
Pb -- Fr_1	221	0.489	0.055
Idh_1 -- Fr_1	240	0.470	0.035
Fr_1 -- W_1	180	0.472	0.052
Idh_1 -- W_1	180	0.484	0.055

References

Griffin, J. D., and R. G. Palmer. 1987. Inheritance and linkage studies with five isozyme loci in soybean. Crop Sci. 27: 885-892.

Hunter, R., and C. Markert. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gel. Science 125:1294 - 1295.

Rennie, B. D., M. L. Thorpe, and W.D. Beversdorf. 1989. A procedural manual for the detection and identification of soybean (*Glycine max* (L.) Merr.) isozymes using the starch gel electrophoretic system. OAC Publication 1889.

Suiter, K.A., J.F. Wendel, and J.S. Case. 1983. Linkage-1: a Pascal computer program for the detection and analysis of genetic linkage. J. Hered. 74: 203-204.

Tachibana H., B. K. Voss, and W. R. Fehr. 1987. Registration of BSR 101 soybean. Crop Sci. 27: 612.

Wendel, J. F., and N. F. Weeden. 1989. Visualization and interpretation of plant isozymes. *In* D.E. Soltis and P.S. Soltis [eds.]. Isozymes in plant biology. 42-72. Dioscorides Press. Portland, OR.

USDA/ARS FIELD CROPS RESEARCH
IOWA STATE UNIVERSITY
Department of Agronomy
Ames, IA 50011

Isolation of Four Independent Monogenically Inherited Male-Sterile Mutants in Soybean

Introduction

Male-sterile, female-fertile mutants refer to mutations affecting male reproductive function, whereas female reproduction is usually complete or slightly reduced. Male sterility in higher plants has been extensively reviewed by Kaul (1988). Male-sterile mutations have been excellent materials for plant breeding, genetics, reproductive biology, and molecular biology studies. Male-sterile mutants have been used in soybean breeding programs to produce hybrid seeds (Lewers, 1996).

Male-sterile mutations in soybean have been reviewed by Palmer et al. (1992). Seven independent loci, *ms1* to *ms 6* and an unknown male-sterile mutation that is non-allelic to *ms1* to *ms 6*, have been described (Jin et al., 1996; Palmer et al., 1992). The *ms1* to *ms 4* alleles were spontaneous mutations in breeding populations, while the *ms5* allele was identified in the neutron-irradiated cultivar Essex (Buss, 1983). Seven independent *ms1* mutations, three independent *ms3* mutations and two independent *ms4* mutations are known in soybean (Palmer et al. 1992).

Four independent new male-sterile mutations (NMM) suspected to be heritable were identified at maturity in 1994 at Bruner farm near Ames, Iowa. The male-sterile plants were green bearing very few pods at harvest time. One NMM, NMM -1, was isolated in F3 plants originally designed for allelism test at the *Mdh1-n* (malate dehydrogenase 1 null) locus in the cross of T261 (*k2 Mdh1-n*) X T323 (*y20 Mdh1-n*). NMM-2 and NMM-3, were isolated in recombination studies involving the *y20 Mdh1-n* (yellow foliage and malate dehydrogenase 1 null) and the *k2* (tan saddle seed coat) loci derived from crosses of T317 X T239 and T325 X L67-3483. NMM-4 was isolated in F2 plants designed for allelism tests at the *Mdh1-n* locus in the cross of T261 (*k2 Mdh1-n*) X PI 567.630A (*Mdh1-n*). The objectives were : (1) to conduct progeny tests with the four NMMs to determine whether these putative male-sterile mutations were heritable, and (2) to determine how many genetic loci were involved in the male-sterile phenotypes in these four NMMs.

Materials and Methods

The experimental materials used in these studies are summarized in Table 1. Genetic Types T239, T261 , Clark isoline L67-3483 and USDA PI 567.630A were obtained from R.L. Nelson, USDA-ARS, Urbana, Illinois.

T317, T323, T325 are yellow foliage (*y20*) and malate dehydrogenase 1 null (*Mdh1-n*) mutants isolated previously (Amberger et al., 1992; Hedges and Palmer, 1992). Standard soybean crossing techniques were used to obtained cross-pollinations (Walker et al., 1979). To verify the success of cross-pollinations, foliage color, flower color, pubescence color and seed coat color were used as morphological markers.

Progeny tests were conducted at the Bruner farm near Ames, Iowa, and at the Iowa State University-University of Puerto Rico Soybean Breeding Nursery, at the Isabela Substation, Isabela, Puerto Rico. Numbers of segregating and nonsegregating families were recorded. Families with less than ten plants that displayed no fertility : sterility segregation in the progeny rows were discarded based on statistical consideration for one-gene Mendelian segregation.

Results and discussions

In fall of 1994, four suspected heritable male-sterile mutations were observed at maturity in a recombination experiment involving the *y20 Mdh1-n* and the *k2* loci in repulsion phase and allelism tests at the *Mdh1-n* locus (Table 2). One F3 family derived from the cross of T261 X T323 segregated about 7 fertile to 1 sterile plants. One F3 family derived from the cross of T317 X T239 segregated about 3 fertile to 1 sterile plants. One F3 family derived from the cross of T325 X L67-3483 segregated complete fertile, partial sterile, and complete sterile plants. The ratio of fertile and partial sterile plant : complete sterile plants fit 3:1 one-gene model. The partial sterile plants were due to a female-sterile mutation in L67-3483, which results in partial sterility in heterozygous plants. One F2 family derived from the cross of T261 X PI 567.630A segregated with fertile, semisterile, and complete male-sterile plants. The ratio of fertile and semisterile plants : complete male-sterile plants fit 3:1 Mendelian model. The semisterile plants in F2 families from the crosses of T261 X PI 567.630A were due to a chromosomal translocation in PI 567.630A (Chen and Palmer, 1996). All the sterile plants in the above mentioned four families bore a few pods with three seeds, suggesting that the sterile phenotypes are female fertile and complete male sterile. The sterile plants were discarded in the segregating families and the fertile plants were harvested individually.

Progeny tests with the F3:4 plants of NMM-1 to NMM-3 and with F2:3 plants of NMM-4 derived from the above four families were conducted in Ames, Iowa, in summer, 1995. Segregation of fertile and complete male-sterile plants were evident in the progeny rows of NMM-1 to NMM-4 (Table 3 and Table 4). Thus, it is suggested that all four male-sterile mutations were heritable. Since the total number of plants per family was small, statistical calculations were not made to estimate the number of genetic loci involved in these nuclear male-sterile mutations. Based upon the pooled data on the fertility : sterility segregation in the segregating families, a 3: 1 Mendelian ratio with the fertility and sterility segregation was evident in all four mutant progeny rows (Table 4). It is suggested that all four male-sterile mutations are monogenically inherited in nature. Thus these male-sterile mutants are maintained in heterozygous condition. Families no. 4 and

9 for NMM-1 and family no. 10 for NMM-2 were maintained. For NMM-3 and NMM-4, families no. 6 and 14 and family no. 7, that did not contain partial sterile or semisterile segregation, were maintained for NMM-3 and NMM-4, respectively (Table 4).

Twenty F4:5 families of NMM-1, NMM-2 and NMM-3 and twenty F3:4 families of NMM-4 derived from the fertile plants were planted in Puerto Rico. Segregation for fertile and male-sterile plants was observed in the progeny rows (Table 5). Thus, it was established that all four male-sterile mutations were heritable. At present, whether these NMMs are allelic to any of the known male-sterile mutations in soybean is still unknown. Further inheritance and allelism tests against the previously reported seven male-sterile mutant loci have been planned.

Reference

Amberger, L. A., R. C. Shoemaker, and R. G. Palmer. (1992). Inheritance of two independent isozyme variants in soybean plants derived from tissue culture. Theor Appl Genet 84: 600-607.

Buss, G. R. (1983). Inheritance of a male-sterile mutant from irradiated Essex soybean. Soybean Genet Newsl 10: 104-108.

Chen, X. F., and R. G. Palmer. (1996). Inheritance and linkage with the *k2* and the *Mdh1-n* loci in soybean. J Hered (in press).

Hedges, B. R., and R. G. Palmer. (1992). Inheritance of malate dehydrogenase nulls in soybean. Biochem Genet 30: 491-502.

Jin, W., H. T. Horner, and R. G. Palmer. (1996). Genetics and cytology of a new genic male-sterile soybean [*Glycine max* (L.) Merr.]. Sexual Plant Reproduction (Submitted).

Kaul, M. L. H. (1988). Male sterility in higher plants. Monographs on Theoretical and Appplied Genetics 10. Springer-Verlag, New York.

Lewers, K. S. (1996). Production, evaluation and utilization of hybrid soybean [*Glycine max* (L.) Merr.]. Ph.D. Diss., Iowa State Univ., Ames, Iowa.

Palmer, R. G., M. C. Albertsen, H. T. Horner, and H. T. Skorupska. (1992). Male-sterility in soybean and maize : developmental comparisons. The Nucleus 35: 1-18.

Walker, A. K., S. R. Cianzio, J. A. Bravo, and W. R. Fehr. (1979). Comparison of emasculation and nonemasculation for hybridization of soybeans. Crop Sci 19: 285-286.

Xianfeng Chen
Reid G. Palmer USDA-ARS

Table 1. Soybean lines used in crosses

Genetic type	Strain no.	Gene symbol	Phenotype
T239	L63-365	*k2* (Urbana)	Green foliage, malate dehydrogenase 1 present, tan saddle seed coat
	L67-3483	*k2* (Columbia 2)	Green foliage, malate dehydrogenase 1 present, tan saddle seed coat
T261	S56-26	*k2* (Columbia 1) *Mdh1-n* (Columbia 1)	Green foliage, malate dehydrogenase 1 null, tan saddle seed coat
T323	CD-1	*y20* (Ames 2) *Mdh1-n* (Ames 2)	Yellow foliage, malate dehydrogenase 1 null, yellow seed coat
T325	CD-3	*y20* (Ames 4) *Mdh1-n* (Ames 4)	Yellow foliage, malate dehydrogenase 1 null, yellow seed coat
	PI 567.630A	*Mdh1-n* (Ames 6)	Green foliage, malate dehydrogenase 1 null, yellow seed coat

Table 2. Isolation of four putative new male-sterile mutants

New male-sterile mutant	Generation	Original cross	Fertile plant	Complete male-sterile plant	Ratio
NMM-1	F3	T261 X T323	51	7	7.29
NMM-2	F3	T317 X T239	44	15	2.93
NMM-3	F3	T325 X L67-3483	34 [a]	9	3.78
NMM-4	F2	T261 X PI 567.630A	16 [b]	5	3.20

[a] Contains partial sterile plants due to a female sterile mutation in L67-3483.

[b] 8 complete fertile plants and 8 semisterile plants due to a chromosomal translocation in PI 567.630A

Table 3. Progeny tests with fertile plants from plants segregating male-sterile plants [a]

New male-sterile mutant	No. homozygous families	No. heterozygous families
NMM-1	31	12
NMM-2	20	18
NMM-3	13	18
NMM-4	4	8

[a] F3:4 families for NMM-1 , NMM-2, and NMM-3; F2:3 families for NMM-4.

Table 4. Fertility : sterility segregations in F3:4 NMM-1, NMM-2 and NMM-3 families and F2:3 NMM-4 families

Family no.	NMM-1		NMM-2		NMM-3 [a]		NMM-4 [b]	
	Fertile	Male- sterile	Fertile	Male-sterile	Fertile	Male-sterile	Fertile	Male-sterile
1	12	7	5	3	23	6	7+ 6 [c]	4
2	28	7	7	2	12	2	5 + 3	3
3	41	18	26	3	21	8	4 + 4	5
4	80	27	42	11	19	3	7 + 6	3
5	11	7	6	4	38	11	25 + 21	24
6	9	5	1	3	43	13	80 + 0	31
7	19	12	44	10	9	3	88 + 0	31
8	9	4	21	11	7	3	11 + 0	4
9	27	10	12	5	14	4		
10	11	6	54	22	15	7		
11	34	10	43	13	8	2		
12	14	2	23	8	28	10		
13			49	12	34	11		
14			8	2	69	18		
15			3	1	18	8		
16			21	5	24	2		
17			24	9	2	1		
18			1	5	5	2		
Total	295	115	390	129	389	114	267	105
Ratio	2.57		3.02		3.41		2.54	
$X^2_{(3:1)}$	2.03		0.00		1.46		2.07	
P (df=1)	0.16		1.00		0.23		0.16	

[a] Contain partial sterile plants due to a female sterile mutation in L67-3483.

[b] Contain semisterile plants due to a chromosomal translocation in PI 567.630A.

[c] Fertile + semisterile plants.

Table 5. Progeny tests with fertile plants from families segregating male-sterile plants [a]

New male-sterile mutant	No. homozygous families	No. heterozygous families
NMM-1	3	17
NMM-2	7	13
NMM-3	5	15
NMM-4	2	18

[a] F4:5 families for NMM-1, NMM-2, and NMM-3; F3:4 families for NMM-4.

IOWA STATE UNIVERSITY
USDA/ARS Field Crops Reseach
Department of Agronomy
Ames, IA 50011

Malate Dehydrogenase 1 Banding Patterns of the Soybean Yellow foliage Mutants

Introduction

Both nuclear and cytoplasmic inherited yellow foliage mutants have been identified in soybean [*Glycine max* (L.) Merr.] (Palmer and Kilen 1987). Among the yellow foliage mutants, five nuclear inherited mutants, Genetic Types T253, T317, T323, T324 and T325, were malate dehydrogenase (MDH) [EC 1.1.1.37] 1 null (*Mdh1-n*) (Amberger et al 1992, Hedges and Palmer 1992). T317 was a somaclonal variant generated from tissue culture (Amberger et al 1992). T323, T324 and T325 were isolated in the progeny rows of *w4-m* mutable line (Hedges and Palmer 1992, Palmer et al 1989). T253 , a spontaneous mutant in T239 background, was recorded originally as a tan saddle (*k2*) and yellow foliage (*y20*) mutant (Palmer 1984). Subsequently, T253 was tested for MDH banding pattern and confirmed to be malate dehydrogenase null which was allelic to the *Mdh1-n* alleles in T317, T324 and T325 (Hedges and Palmer 1992). Thus, T253 is a triple mutant with tan saddle seed coat (*k2*), *Mdh1-n*, and yellow foliage (*y20*) phenotype. No recombination between the *k2* and *y20* loci was detected with T253 crossed with wild type (Palmer 1984). All the above five yellow foliage, malate dehydrogenase null mutants were allelic at *y20* and *Mdh1-n* loci (Amberger et al 1992, Hedges and Palmer 1992). The yellow foliage (*y20*) and *Mdh1* null phenotypes in T317, T323, T324 and T325 always were co-inherited as a single recessive nuclear gene. No confirmed crossovers between the *y20* and the *Mdh1-n* loci have been identified (Amberger et al 1992, Hedges and Palmer 1992).

Eight *Mdh1-n* mutants have been reported at *k2 Mdh1-n y20* chromosomal region in soybean (Amberger et al 1992, Chen and Palmer 1996, Hedges and Palmer 1992, Palmer et al 1989). Two Chinese accessions, USDA germplasm collection PI 567.391 and PI 567.630A, were identified as green foliage *Mdh1-n*. Originally, T261 was recorded as a tan saddle mutant, subsequently, T261 was confirmed to be *Mdh1* null (Chen and Palmer 1996). Doong and Kiang (1987) screened 93 USDA Soybean Genetic Type Collection mutants including T234, T253, and T261. They reported that all the 93 Genetic Type Collections were monozymogramic with six MDH isozyme bands, and found no variant types. The purpose of our research was to

screen the nuclear and cytoplasmic inherited yellow foliage mutants in the USDA Genetic Type Collection for MDH banding patterns and to possibly identify new mutants at the *k2 Mdh1-n y20* chromosomal region in soybean.

Materials and Methods

Soybean mutants screened in this study were obtained from R.L. Nelson, USDA-ARS, Urbana, Illinois (Table 1). Seeds were germinated on germination paper for three days. Fifteen or more seedlings of the heterozygous entries were transplanted to sandbench after being sampled for electrophoresis. The identities of the seeds/seedlings were maintained. Foliage color segregations were confirmed one week after transplanting.

MDH isozyme pattern determination followed the starch gel electrophoresis procedure described by Cardy and Beversdorf (1984a, b). Electrophoretic samples were taken from the cotyledons of the three-day-old seedlings by using a 100-ul micropipette. The samples were stored at -70 ° C until assayed. Starch gels were prepared by using 11.78% starch concentrations with "B" gel and electrode buffer systems (pH 6.5). Gels were run at a constant power of 9.5 W for 5.5 hours in a 4 ° C chromatography cooling chamber.

Results and Discussions

Fourty-seven nuclear inherited and seven cytoplasmic inherited yellow foliage mutants were tested for MDH banding patterns. All these mutants contained *Mdh1* except T234 which was malate dehydrogenase null. Thus, T234 is a yellow foliage and putative *Mdh1-n* mutant. Doong and Kiang (1987) reported that T234 had the same MDH banding pattern as the other 92 Genetic Type collections surveyed. Thus they missed T234 as a malate dehydrogenase null mutant. Doong and Kiang (1987) used slab-gel electrophoretic system which could only resolve six MDH bands. We used starch gel electrophoretic system which is able to resolve up to eight MDH bands. The T234 malate dehydrogenase null mutant was missing the two lowest mobility MDH isozyme bands on our electrophoretic gels.

The yellow foliage phenotype of T253 was similar to that of T317, T253, T323, T324 and T325 which was conditioned by the *y20* recessive nuclear allele. Thus, it is highly likely that the yellow foliage phenotype of T234 is determined by *y20* allele instead of the *y15* allele. The gene symbol *y15* was assigned to T234, but we have not been able to find any written record of the genetic studies. Further allelism and inheritance studies with T234 should be done to test the hypothesis that T234 is a *y20 Mdh1-n* mutant.

References

Amberger L. A., R C. Shoemaker, and R. G. Palmer. (1992). Inheritance of two independent isozyme variants in soybean plants derived from tissue culture. Theor. Appl. Genet. 84: 600-607.

Cardy B. J., and W. D. Beversdorf. (1984a). A procedure for the starch gel electrophoretic detection of isozymes in soybean [*Glycine max* (L.) Merr.]. Dep Crop Sci Tech Bull

119/8401. University of Guelph, Ontario, Canada.

Cardy B. J., and W. D. Beversdorf. (1984b). Identification of soybean cultivars using isoenzyme electrophoresis. Seed Sci. Technol. 12: 943-954.

Chen X. F., and R. G. Palmer. (1996). Inheritance and linkage with the *k2* and *Mdh1-n* loci in soybean. J. Hered. (in press).

Doong J. Y. H. , and Kiang Y. T. (1987). Cultivar identification by isozyme analysis. Soybean Genet Newsletter 14: 189-226.

Hedges B. R., R. G. Palmer. (1992). Inheritance of malate dehydrogenase nulls in soybean. Biochem Genet. 30: 491-502.

Palmer R. G. (1984). Pleiotropy or close linkage of the two mutants in soybeans. J. Hered. 75: 445-447.

Palmer R. G., and T. C. Kilen. (1987). Qualitative genetics. In Wilcox JR (ed), Soybeans: Improvement, production, and uses, 2nd ed. Am. Soc. Agron, Madison, WI, pp 135-209.

Palmer R. G., B. R. Hedges, R. S. Benavente, and R. W. Groose. (1989). The *w4* mutable line in soybean. Dev. Genet. 10: 542-551.

X. F. Chen
R. G. Palmer -USDA

Soybean yellow foliage mutants screened for malate dehydrogenase 1 patterns

Genetic Type *	Gene symbol	No. of seeds tested	*Mdh1* banding pattern
T102	*y4*	3	Present
T116H	*y5*	30	Present
T134	*y5*	3	Present
T135	*y9*	3	Present
T136	*y6*	3	Present
T138	*y7 y8*	3	Present
T139	*y3*	3	Present
T143	*y3 y7 y8*	3	Present
T144	*y7 y8*	3	Present
T160	-	3	Present
T161	*y10*	3	Present
T162	*y17*	3	Present
T164	-	3	Present
T218M	*Y18-m*	3	Present
T219H	*y11*	30	Present
T220	-	3	Present
T221	-	3	Present
T223	-	3	Present
T224	-	3	Present
T225H	*y18*	30	Present
T225M	*Y18-m*	3	Present
T226	-	3	Present
T227	-	3	Present
T229	*y14*	3	Present
T230	*y13*	3	Present
T231	-	3	Present
T232	-	3	Present
T233	*y12*	3	Present
T234	*y15*	30	**Null**
T249H	*p1*	30	Present
T250H	-	30	Present
T252	-	3	Present
T254	-	3	Present
T257H	*y16*	30	Present
T265H	*y19*	30	Present
T270H	*y22*	30	Present
T275	*cyt-Y2*	6	Present
T278M	*cyt-Y3*	18	Present
T283	-	3	Present
T288	*y23*	3	Present
T314	*cyt-Y4*	3	Present
T315	*cyt-Y5*	3	Present
T316	*cyt-Y6*	3	Present
T319	*cyt-Y7*	3	Present
T320	*cyt-Y8*	3	Present
PI 84619	-	3	Present
PI 507019	-	3	Present
PI 507429	-	3	Present
PI 507430	-	3	Present
PI 507477	-	3	Present
PI 507480	-	3	Present
PI 158252	-	3	Present
LG 87-6108	-	15	Present
LG 87-6047	-	15	Present

* PI and LG87 - designations refer to yellow foliage mutants, but they have not been added to the Genetic Type Collection.

UNIVERSITY OF ILLINOIS
Department of Crop Sciences
1101 W. Peabody Dr.
Urbana, IL 61801

Genetic Analysis of the Response of the Soybean Cultivar Asgrow A4715 to *Fusarium solani.*

Introduction

Resistance to *Fusarium solani* [(Mart.) Appel & Wollenw. emend. Snyd. & Hans.] is important because sudden death syndrome (SDS) has been the discovered in several areas of soybean [*Glycine max.* (L.) Merr] production (Rupe, 1992). Resistance and a delay in planting date (Hershman et al., 1990) appear to be the only economical method of disease reduction. Understanding the genetics of the resistance to SDS in soybean will be beneficial to breeders for future SDS control.

Variation in cultivar reaction is common between locations and years. Rupe et al. (1991) showed 'Leflore' (Hartwig et al., 1985) and Asgrow 'A5474' (Shannon and Schillinger, 1989) to be moderately resistant cultivars, where previously they were characterized as susceptible. Stephens et al. (1993a,b) found different reactions for Asgrow 'A4715' (Asgrow 'A5474' X Asgrow 'A4595') in field plots, microplots and the greenhouse. Asgrow A5474, Leflore, and Asgrow A4715, have very similar parentage, and may have the same source of resistance. Variation within the *F. solani* population suggests multiple races of the disease. Lim and Jin (1991) identified at least five races based on differential responses of ten genotypes. Multiple races of *F. solani* suggest that many genes for resistance to SDS may exist in soybean.

Stephens and Nickell (1993) identified a single dominant nuclear gene (*Rfs*) that controls the resistance to *F. solani* in cultivar Ripley (Cooper et al., 1990). They evaluated crosses of Ripley with the cultivar Spencer (Wilcox et al., 1989) in a greenhouse environment using an inoculation technique very similar to the technique described by Lim (1991). The greenhouse inoculation technique that uses an *F. solani* mycelium fragment suspension caused little SDS symptom development on the cultivar Ripley, but cultivars, Asgrow A4715 and Spencer, developed severe foliar SDS symptoms. Our objective in this study was to use this greenhouse inoculation technique to determine the inheritance of the response of the soybean cultivar Asgrow A4715 to *F. solani* isolate ST90.

Materials and Methods

Crosses were made in the summer of 1993 at the Agronomy Plant Pathology South Farm, Urbana, IL. Cultivars, Ripley and Spencer, were crossed reciprocally to the cultivar Asgrow A4715. During the 1993-94

winter, F_1 plants were grown in the greenhouse at Urbana, IL, to produce F_2 seed. Additional F_1 plants were also grown during the summer of 1994. F_2 plants were grown in the summer of 1994, and single plants were harvested at maturity from each F_2 population to produce $F_{2:3}$ families. Crosses were determined to be valid by following phenotypic markers from the parental lines in each generation of the progeny.

Plants from the F_1, F_2, and F_3 along with the parental lines were inoculated at planting with an *F. solani* mycelium suspension. Three 0.5 cm^2 plugs of *F. solani* were used to inoculate 100 ml of a soybean seed broth media maintained for 10 d at 21 C°. The soybean seed broth media was prepared as described by Willmot and Nickell (1989). After the incubation period, the mycelium suspension was blended using a kitchen blender for 2 min. After an initial mycelium fragment concentration was determined, the mycelium fragment solution was diluted to a concentration of 2.0×10^4 fragments per ml and amended with 10 g per L of carboxy methyl cellulose before inoculation.

Seeds from each generation and parental lines were placed in 2.5 cm deep holes in 15 cm steamed clay pots that contained a 1:1 steamed mixture of soil to sand. Each pot was inoculated with 30 ml of a mycelium suspension. Each seed received 3 ml of the inoculum. After inoculation a small portion of soil was used to cover the inoculated seeds. After seedlings emerged, pots were thinned to six uniform seedlings per pot.

The *F. solani* isolate was ST90; the same isolate used by Stephens and Nickell (1993). The isolate was maintained on 1/3 strength potato dextrose agar with 50 ug L^{-1} tetracycline (Stephens and Nickell, 1993) in a 21° C incubator. After inoculation pots were maintained, in the greenhouse, at 18°-27° C with a 14 hr photoperiod. Each pot received 150 ml of water twice daily and was fertilized weekly with a 150 ml liquid fertilizer solution that contained 98 mg N, 89 mg P_2O_5, 85 mg K_2O, 0.12 mg chelated Cu, Mn, and Zn, 0.05 mg B and 0.24 mg chelated Fe L^{-1}. This solution was the result of combining 1.56 g of Ra-pid-gro Plant Food (Chevron Chemical Co., San Francisco, CA) and 1.54 g Peter's Fertilizer (W.R. Grace & Co., Fogelsville, PA).

Seedlings were classified for their foliar reaction to *F. solani* during the V3 (Fehr et al., 1971) stage of growth at approximately 21 d after inoculation. Each plant was classified as resistant or susceptible based on the observed reaction of the parental types in all generations, and $F_{2:3}$ families were then classified as resistant, heterogeneous, or susceptible. The exact day of classification was based on the foliar symptom development in the parental lines or at the time of greatest difference between the resistant and susceptible parents. After foliar evaluation, plants that developed no foliar symptoms were removed and the taproots of these seedlings were evaluated to ensure they were infected. Plants without taproot necrosis were treated as escapes and not considered in the analysis.

Results and Discussion

Asgrow A4715 and Spencer seedlings began to show foliar symptoms at the unifoliolate stage of growth, but did not develop severe enough symptoms for classification until the first trifoliolate stage of growth. Some Ripley seedlings also developed some foliar symptoms at this time, but generally they were less severe than the symptoms that developed with Asgrow A4715 and Spencer. Ripley seedlings were also unique in the fact that the unifoliolate leaves developed little or no chlorosis. When chlorosis did appear, it was very mild in comparison with Asgrow A4715 and Spencer. Plants were classified as resistant or susceptible based on the described parental reactions at approximately 21 d after inoculation or at the V2 or V3 growth stage. The data from reciprocal crosses were combined because of no apparent cytoplasmic effects (Table 1). The evaluated F_2 and F_3 plants from the crosses of Ripley x A4715 had low probabilities for goodness of fit for a single dominant gene model (Table 1). The error observed with the classification of parental types is probably responsible (Table 1). The F_3 family segregation, where the least amount of error was expected to occur, fit a 1:2:1 ratio of resistant to heterogeneous to susceptible. When resistant and homogeneous families were grouped together (3:1 segregation of $F_{2:3}$ families) from the reciprocal crosses, the single dominant nuclear gene model fits very well. Some resistant families were misclassified into the heterogeneous class to cause the lower probability that occurred with fitting to the 1:2:1 model. These data confirm the results presented by Stephens and Nickell (1993) that Ripley carries a single dominant nuclear gene (*Rfs*) for resistance to *F. solani* with no cytoplasmic effects.

Asgrow A4715 and Spencer both developed severe foliar symptoms with the ST90 isolate used in evaluation, and the data from each generation, fit with the expected susceptible reactions (Table 1). Classification error occurred in the evaluation, a few plants seemed resistant, but this error seems similar with that of the parental types. Both cultivars appear to have the recessive (*rfs*) allele at the locus that is responsible for the resistance in the cultivar Ripley. Classification of plants into resistant and susceptible classes using the inoculation technique is difficult and influenced by environmental conditions in the greenhouse. This source of error makes elucidation of the inheritance of SDS resistance difficult and illustrates the important role the environment plays in determining the severity of SDS under field condition.

The original objective of this study was to determine the inheritance of the response of Asgrow A4715 to isolate ST90 (Stephens et al., 1993b). Asgrow A4715 is susceptible to all other isolates. Asgrow A5474 and Leflore have shown variable reactions to *F. solani* in the field (Rupe et al., 1991). These two cultivars and Asgrow A4715 have similar pedigrees and we can hypothesize that they may have the same genetic source for this variable reaction. Lim and Jin (1991) found reactions that suggest multiple races of *F. solani* based on the differential reactions of 10 genotypes. Races of the fungus may explain this variable reaction, but this is yet to be determined. Another question is whether resistance to *F. solani* is quantitative in nature. In field

studies, the different degrees of disease severity that develop in the soybean population suggest this also may be true. We know that resistance in the cultivar Ripley is controlled by a single dominant gene but that may not be the case with other soybean cultivars. Further studies of the genetics of resistance to *F. solani* should be completed to determine how *F. solani* affects other genotypes, and to determine if races of the fungus are in the *F. solani* population.

Table 1. Reaction of F_1, F_2 and $F_{2:3}$ families from the crosses of Ripley x Asgrow A4715 and Spencer x Asgrow A4715 to inoculation with *Fusarium solani*.

Cross[1]	Res.[2]	Het.[3]	Susc.[4]	Theor. Ratio	Chi-square	Prob.
	----------no. plants----------					
Ripley x Asgrow A4715						
F_1	10		0			
F_2	157		109	3:1	36.22	0.0001
$F_{2:3}$ families	7	29	14	1:2:1	3.24	0.2000
$F_{2:3}$ families[5]	36		14	3:1	0.24	0.6242
F_3	304		286	5:3	30.31	0.0001
Ripley	97		19			
Asgrow A4715	19		94			
Spencer x Asgrow A4715						
F_1	0		11			
F_2	54		200			
$F_{2:3}$ families	0	0	50			
$F_{2:3}$ families[5]	0		50			
F_3	39		259			
Asgrow A4715	23		80			
Spencer	29		79			

[1] Reciprocal crosses were combined since no cytoplasmic effects were observed.
[2] Observed resistant plants and families.
[3] Observed heterogeneous families.
[4] Observed susceptible plants and families.
[5] Resistant and heterogeneous families combined.

References

Cooper, R. L., R. J. Martin, B. A. McBlain, R. J. Fioritto, S. K. St. Martin, A. Calip-Dubois, and A. F. Schmitt-henner. 1990. Registration of 'Ripley' soybean. Crop Sci. 30:963.

Fehr, W. R., C. E Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stage development description for soybeans,*Glycine max* (L.) Merr. Crop Sci. 11:929-931.

Hartwig, E. E., L. D. Young, L. D., and S. C. Edwards Jr. 1985. Registration of 'Leflore' soybean. Crop Sci. 25:1128-1129.

Hershman, D. E., J. W. Hendrix, R. E. Stuckey, P. R. Bachi, and G. Henson. 1990. Influence of planting date and cultivar on soybean sudden death syndrome in Kentucky. Plant Dis. 74:761-764.

Lim, S. M. 1991. A technique for inoculating soybeans in the greenhouse with *Fusarium solani*. (Abstr.) Phytopathology 81:1238.

Lim, S. M. and H. Jin. 1991. Pathogenic variation in *Fusarium solani* isolated from soybeans with sudden death syndrome symptoms. (Abstr.) Phytopathology 81:1236.

Rupe, J. C. 1992. Nature and management of soilborne fungal diseases of soybean. p.196-205. *In* L.G. Copping, M. B. Green, and R.T. Rees (ed.) Pest Management In soybean. Elsevier Applied Science Publ. London, England and New York, New York.

Rupe, J. C., E. E. Gbur, and D. M. Marx. 1991. Cultivar responses to sudden death syndrome of soybean. Plant Dis. 75:47-50.

Shannon, J. G., and J. A. Schillinger. 1989. Registration of 'A5474' soybean. Crop Sci. 29 : 1091.

Stephens, P. A. and C. D. Nickell. 1993. Genetic analysis of resistance to *Fusarium solani* in soybean. Crop Sci. 33:929-930.

Stephens, P. A., C. D. Nickell, and S.M. Lim. 1993a. Sudden death syndrome development in soybean cultivars differing in resistance to *Fusarium solani*.. Crop Sci. 33:63-66.

Stephens, P. A., C. D. Nickell, C. K. Moots, and S. M. Lim. 1993b. Relationship between field and green-house reactions of soybean to *Fusarium solani*. Plant Dis. 77:163-166.

Wilcox, J. R., M. T. Roach, and T. S. Abney. 1989. Registration of 'Spencer' soybean. Crop Sci. 29:830-831.

Willmot, D. B., and C.D. Nickell. 1989. Genetic analysis of brown stem rot resistance in soybean. Crop Sci. 29: 672-674.

Daniel L. Heavner

Cecil D. Nickell

F. L. Kolb

PIONEER HI-BRED INT'L. INC.[1]
UNIVERSITY OF ILLINOIS [2]
2118 County Rd. 1600N
ST. Joseph, IL 61873

Genetic Resistance to *Fusarium solani* in Pioneer Brand 9451 Soybean

Introduction

Sudden death syndrome (SDS) is a fungal disease of soybean found in the southern Midwest. Sudden death syndrome was first reported in Arkansas in 1971 by H. J. Walters and by the mid 1980's had become recognized as an economically important disease in Illinois, Indiana, Missouri, Mississippi, and Kentucky (Hershman et al., 1990). A pathogenic strain of *Fusarium solani* has been identified as the cause of the leaf necrosis and cortical rot associated with SDS (Roy et al., 1989; Rupe, 1989). A 1985 study in Arkansas showed SDS resistant or tolerant cultivars to have a substantial yield advantage over cultivars rated as susceptible (Hirrel, 1986). Identifying and incorporating sources of SDS resistance has become an important priority of both public and private soybean breeding programs.

In a study measuring the reaction of 12 soybean cultivars to *Fusarium solani*, cultivar Ripley remained nearly symptom free while the highly susceptible cultivar Spencer developed severe leaf necrosis. Ripley was subsequently shown, by genetic analysis, to possess a single dominant gene, *Rfs*, for resistance to infection by *Fusarium solani*. (Stephens et al., 1993a).

In the 1992 hill plot study, the cultivar Pioneer Brand 9451 (9451) was identified as having a level of resistance to *Fusarium solani* comparable to that of Ripley (Stephens et al., 1993b). The objectives of this study were to determine the genetic mode of resistance to *Fusarium solani* found in 9451 and to study the allelic relationship to the *Rfs* gene found in Ripley.

Materials and Methods

In January 1993, crosses and their reciprocals were made between 9451 and Ripley and between 9451 and Spencer. F_1 seeds of the four crosses, 9451 x Spencer, Spencer x 9451, 9451 x Ripley, and Ripley x 9451, were grown in the field at St. Joseph, IL during the summer of 1993. Crosses between 9451 and Ripley were confirmed in the field based on genetic markers in the parent cultivars for flower color and pubescence color.

In the summer of 1994, F_2 seeds from each cross and seed of the parent cultivars were planted in the field at St. Joseph, IL. The plot area was inoculated before planting with *Fusarium solani* infested white sorghum (*Sorghum vulgare)* at the rate of one sorghum grain per six cm^2 of surface area. The inoculant was broadcast over the plot area and incorporated to a depth of three inches using a garden tiller.

Seed from the bulked F_1's of the 9451 x Ripley and Ripley x 9451 crosses were planted at the rate of 360 seeds in 15 m of row. Seed from three F_1 plants (120 seeds per plant) of the 9451 x Spencer and Spencer x 9451 crosses were represented by three 5 m rows of each cross. Plots were bordered by rows of the parent cultivars (9451, Ripley, and Spencer). Irrigation was applied at the beginning of R1 growth stage to provide additional moisture (Fehr et al, 1971). The populations and parents were checked closely for symptoms every 7 to 10 days. No SDS symptoms appeared on any plants in the study. At maturity, all seed bearing plants were harvested separately to produce F_3 families

A second attempt was made to evaluate the F_2 populations in the St. Joseph, IL greenhouse soilbed in January through March of 1995. The 9451 x Spencer cross and the reciprocal were confirmed based on segregation for hilum color in the $F_{2:3}$ families, which allowed bulking of the F_2's. On 19 January, 1995, each of the four populations was planted in eight random subplots. Each subplot was 75 cm long and contained 25 seeds. The three parents were alternated with the population sub-plots in 25 cm rows planted with 10 seeds each. The soilbed was laid out with eight rows 3.75 m in length with rows 60 cm apart. Each row contained one subplot of each population and one plot of each of the three parent cultivars. A drip irrigation system supplied water sufficient to maintain a high soil moisture level throughout the experiment. Greenhouse temperatures ranged from 15° C at night to 20-30° C during the day. Day-length was artificially lengthened to 20 hours for the first week after planting and then decreased by one hour per week.

Seedlings were inoculated at 20 d after planting at growth stage V2. The inoculation technique was as described by Stephens et al. (1993a) with some modification. Seedlings were not transplanted before inoculation and the *Fusarium solani* was cultured on white sorghum rather than oats (*Avena sativa).* Inoculum was 12-14 d old and cultured from plates of actively growing *Fusarium solani.* Two grains of *Fusarium solani* infested white sorghum were placed next to the taproot of each seedling, about 2 cm below the soil surface. Two methods were used to apply the inoculum. On odd numbered rows, a knife blade was slid downward along the seedling stem and taproot to a depth of approximately 2 cm. Two grains of *Fusarium solani* infested sorghum were dropped in and the slot closed. On even numbered rows, a spoon was used to pull the soil back from several seedlings at a time and two grains of inoculum were placed next to the taproot of each seedling. The soil was then replaced and gently tamped. Care was taken to prevent root injury although some root wounding did occur as the soil was moved away from the taproot of the seedlings in both methods. Plots were observed daily and individual plants were scored for severity at 44 d after planting. The plants were scored on a 1 to 4 scale with: 1 = no symptoms, 2 = slight symptoms, 3 = moderate, and 4 = severe symptoms. At 61 d after planting the plants were given a final foliar score using the same 1-4 scale. Based on the mean score and standard deviation of the parent cultivars (Table 1), the F_2 plants with scores of 1 or 2 were classified as resistant and those with scores of 3 or 4 were classified as susceptible. There were 306 F_2 plants evaluated for the 9451 x Spencer and Spencer x 9451 crosses and 274 F_2 plants evaluated for the 9451 x Ripley and Ripley x

9451 crosses.

The $F_{2:3}$ single plants derived from the F_2 bulks grown in the summer of 1994 were evaluated in the University of Illinois greenhouse beginning in January 1995. Forty-two $F_{2:3}$ families from each of the four populations were evaluated by planting 10 seeds from each family in a 15 cm steamed clay pot containing sterilized 1:1 sand to soil mix. Seeds were planted 27 January 1995 and thinned to 7 seedlings per pot at emergence. Seedlings were inoculated with 2 grains of *Fusarium solani* infested white sorghum per seedling at the V1 growth stage. A second experiment was begun on 24 March 1995 with the same set for 42 families from each population. Pots were planted with 10 seeds but not thinned and the inoculum rate was reduced to one grain of *Fusarium solani* infested sorghum per seedling. Seedlings were scored for incidence and severity on the same basis as the first set.

The *Fusarium solani* isolate used in this study was initially cultured from SDS symptomatic plants of cultivar Asgrow A3427 found in a field near Monticello, Illinois, hence its designation 'Monticello isolate . The isolate was maintained on 1/3 strength potato dextrose agar and reisolated periodically from greenhouse and field grown plants of Spencer.

Temperatures in the University of Illinois greenhouse were maintained at 15-18° C during the day and 10° C. at night. The photoperiod was artificially lengthened with metal halide lighting to 14 hours per day. Pots received 150 ml of water twice daily from an automatic drip-irrigation system.

Pots were checked for symptoms every two to three days until symptoms appeared. Seedlings in each pot were scored individually on a 1 to 4 scale (1 = no symptoms, 2 = slight symptoms, 3 = moderate symptoms, and 4 = severe symptoms) when differentiation between the parental checks within a bench section appeared to be at a maximum.

Conclusion

Analysis of reaction to inoculation for the $F_{2:3}$ families (Table 2) and F_3 plants suggest Pioneer Brand 9451 may carry a gene for resistance to *Fusarium solani* that is different from the *Rfs* gene identified by Stephens et al. (1993a) in Ripley. The possibility also exists for 9451 to carry two or more genes for resistance to the pathogen. However, the data are inconsistent and need to be confirmed by further investigation.

Both Pioneer Brand 9451 and Ripley are highly resistant to *Fusarium solani* in field trials and on production acreage for the past three years. In three years of plot studies, the only SDS symptoms noted on either cultivar occurred when the inoculant was planted with the seed. In the same trials, Spencer and other susceptible cultivars showed severe symptoms of SDS and significant yield loss.

Table 1. Reaction of F_2 plants of the populations and parent cultivars to seedling inoculation with *Fusarium solani* infested sorghum in the St. Joseph, IL greenhouse soilbed.

	Parent score[c]		F_2 Plants [a]						
		Std	Observed ratio		Expected ratio		Chi		Ratio
Cultivar / Population[b]	Mean	dev.	R	S	R_E	S_E	square	P[d]	tested
			----------number ----------						
Pioneer Brand 9451	2.4	1.0							
Ripley	2.3	1.2							
Spencer	3.3	1.1							
Pioneer Brand 9451 x Spencer			89	62	113	37.5	21.2	0	3 : 1
Pioneer Brand 9451 x Ripley			62	99	151	10.1	838.5	0	15 : 1

a Plants were classified as resistant (R) if scored 1 = no symptoms or 2 = slight symptoms and susceptible (S) if scored 3 = moderate symptoms or 4 = severe symptoms.

b Reciprocals combined.

c Mean score of parent cultivars

d Probability of a greater chi-square due to chance deviation.

Table 2. Reaction of $F_{2:3}$ families from the populations Pioneer Brand 9451 x Spencer and Pioneer Brand 9451 x Ripley (reciprocals combined) to seed inoculation with *Fusarium solani* infested sorghum in the University of Illinois greenhouse.

Population	Parent score		$F_{2:3}$ Families[a]								
			Observed ratio			Expected ratio			Chi		Ratio
	Mean	Std. Dev	R	H	S	R_E	H_E	S_E	square	P^b	tested
			number								
Pioneer Brand 9451	1.7	0.8									
Ripley	1.8	0.7									
Spencer	2.8	1.0									
Pioneer Brand 9451 x Spencer			52	87	29	42.0	84.0	42.0	6.51	0.04	1 : 2 : 1[c]
Pioneer Brand 9451 x Spencer			139		29	126.0		42.0	5.37	0.02	3 : 1[d]
Pioneer Brand 9451 x Spencer			139		29	157.5		10.5	35.46	0.00	15 : 1[e]
Pioneer Brand 9451 x Ripley			63	88	17	73.5	84.2	10.5	5.67	0.06	7 : 8 : 1[f]
Pioneer Brand 9451 x Ripley			63	88	17	115.7	42.1	10.5	78.15	0.00	11 : 4 : 1[g]
Pioneer Brand 9451 x Ripley			151		17	157.5		10.5	4.29	0.04	15 : 1[h]

a Families were classified as resistant (R) if less than two plants in a family were scored as 3 = moderate symptoms or 4 = severe symptoms. Families were classified as susceptible (S) if less than two plants were scored as 1 (= no symptoms) or 2 (= slight symptoms). All remaining families were classified as heterogeneous (H).

b Probability of a greater chi-square due to chance deviation.

c The expected segregation ratio assuming a single gene for resistance in 9451 is 1:2:1.

d The 3:1 ratio was derived by combining the segregation and resistant families into the resistant class.

e The expected ratio assuming two genes for resistance in 9451 is 15:1.

f The 7:8:1 ratio assumes 9451 and Ripley each carry a different gene for resistance.

g The 11:4:1 ratio assumes families segregating 15:1 are included in the resistant class.

h The final 15:1 ratio assumes two genes for resistance in Pioneer Brand 9451 different from the *Rfs* gene in Ripley.

References

Fehr, W. R., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stages of development descriptions for soybeans, Glycine max (L.) Merr. Crop Science 11:929-931.

Hershman, D. E., J. W. Hendrix, R. E. Stuckey, P. R. Bachi, and G.Henson. 1990. Influence of planting date and cultivar on sudden death syndrome in Kentucky. Plant Disease 74:761-766.

Hirrel, M. C. 1986. Sudden death syndrome of soybean: new insight into its development. p. 95-105. In Proceedings. Soybean Seed Conference, 16th, American Seed Trade Association, Washington, D.C.

Killebrew, J. F., K. W. Roy, G. W. Lawrence, K. S. McLean, and H. H. Hodges. 1988. Greenhouse and field evaluation of *Fusarium solani* pathogenicity to soybean seedlings. Plant Disease 72:1067-1070.

Roy, K. W., G. W. Lawrence, H. H. Hodges, K. S. McLean, and J. F. Killebrew. 1989. Sudden death syndrome of soybean: *Fusarium solani* as incitant and relation of *Heterodera glycines* to disease severity. Phytopathology 79:191-197.

Rupe, J. C. 1989. Frequency and pathogenicity of *Fusarium solani* recovered from soybeans with sudden death syndrome. Plant Disease 73:581-584.

Rupe, J. C., E. E. Gbur, and D. M. Marx. 1991. Cultivar responses to sudden death syndrome of soybeans. Plant Disease 75:47-50.

Stephens, P. A., C. D. Nickell, and F. L. Kolb. 1993a. Genetic analysis of1971 resistance to *Fusarium solani* in soybean. Crop Science 33:929-930.

Stephens, P. A., C. D. Nickell, and S. M. Lim. 1993b. Sudden death syndrome development in soybean cultivars differing in resistance to *Fusarium solani*. Crop Science 33:63-66.

G. A. Ringler[1]

C. D. Nickell[2]

SOUTHERN ILLINOIS UNIVERSITY
Department of Plant and Soil Science
Molecular Science Program
Carbondale, IL 62901.

Genetic Analysis of Resistance to Manganese Toxicity in Soybean Using Molecular Markers.

Manganese toxicity is a problem associated with soybeans grown on acidic soils that may limit soybean yields in the Southern US. Every year farmers spend thousands applying lime to reduce the pH level in acid soils and thereby reduce the effects of Mn toxicity. However, the application of lime occurs weather permitting. Soybean cultivars with improved manganese toxicity resistance might protect yields in acid soils, particularly in years when Spring rainfall precludes the application of lime. Many studies have shown that soybean cultivars vary in plant growth response to toxic levels of Mn in solution (Carter et al., 1975; Henan and Campbell, 1980, Ohki et al., 1987). The molecular map of Essex and Forrest soybean makes it possible to map manganese tolerance Quantitative Trait Loci (QTL) in adapted germplasm. Since Essex and Forrest contrast for their resistance to Mn toxicity (Carter et al., 1975) and SDS resistance we can use the ExF RIL population and molecular map for comparisons with Sudden Death Syndrome resistance QTL (Hnetkovsky et al., 1996; Chang et al., 1996). Forrest is resistant to SDS but sensitive to Mn toxicity while Essex is resistant to Mn toxicity but sensitive to SDS.

Materials and Methods

Mn resistance was analyzed in Essex, Forrest and their F_2 derived $F_{5:9}$ progenies. Traits were determined in two experiments (four replicates per experiment with plants grown in hydroponics for 28 days containing 125 µM Mn, the toxic concentration. Traits measured included chlorosis (Mg1 at 14 days, Mg6 at 28 days), wilt (Mg5), height (Mg2), shoot weight (Mg3=fresh), (Mg7=dry) and root weight (Mg4=fresh), (Mg8=dry). The relative contributions of individual genes to total manganese tolerance was estimated by testing for associations with particular chromosomal segments by Mapmaker/ QTL 1.1 and with particular markers by one way ANOVA.

Results

One hundred and thirty-five markers covering more than 2000 cM, including ten linkage groups, were tested for association. Twenty markers were associated ($P < 0.05$) with at least one Mn trait. The twenty markers identified at least at least six independent QTL conditioning Mn resistance as judged by map position (at $P < 0.005$, Table 1). Three of these QTL defined by; OC01, (QTL N), OEO4 and OE02 (QTL 2G) and OG13 (QTL 1G) were less than 15 cM from the map positions of SDS resistance QTL (Hnetkovsky et al., 1996; Chang et al., 1996).

Markers OCO1, OF04 and OO04, that map to a 20 cM interval on linkage

group N (Hnetkovsky et al., 1996), were associated significantly with most Mn toxicity traits including; chlorosis, wilt, height, root and shoot weight. The QTL explained up to 36% of the trait variability (Table 1). In contrast the SDS QTL on linkage group G (Chang et al., 1996) was only associated with chlorosis (OE02) or chlorosis, wilt and shoot weight (OG13). For each of these QTL the beneficial allele for Mn resistance was derived from Essex whereas the SDS resistance allele was derived from Forrest. Therefore, QTL for Mn toxicity and SDS resistances are in repulsion.

Three unlinked markers found associated with Mn toxicity resistance are not associated with SDS resistance; OU15 was associated with the chlorosis and height Mn toxicity traits. OC10 was associated with the wilt Mn toxicity trait. OC11 was associated with the wilt, chlorosis, root and shoot dry weight Mn toxicity traits. The QTL explained up to 36% of the trait variability (Table 1). The beneficial alleles for these QTL were derived from Essex.

Jointly the QTL can explain about 75% of each trait. Therefore, resistance to Mn toxicity in the Essex soybean is controlled by 3-4 major QTL. Since SDS resistance and Mn toxicity resistance QTL were in repulsion, either linkage exists or the physiological bases of the Mn and SDS responses share some common elements. Leaf Mn deficiency has been reported to be associated with increased SDS severity in the field (Rupe et al., 1994). Marker saturation and subline population analyses may allow the QTL to be separated. Markers capable of distinguishing the QTL could be used to break linkage between SDS resistance and Mn toxicity susceptibility. The greenhouse Mn resistance reaction may predict partial SDS resistance better than assays including *Fusarium solani*, the incitant of SDS (see Torto et al., 1996).

References

Brown J. C. and W. E. Jones. 1974. Differential response of oats to manganese stress. Agro. J. 66: 624-626.

Carter, O. G., I. A. Rose, and P. F. Reading. 1975. Variation in susceptibility to manganese toxicity in 30 soybean genotypes. Crop Sci. 15: 730-732.

Chang S. J. C., T. W. Doubler, V. Kilo, R. Suttner, J. Klein, M. E. Schmidt, P. T. Gibson and D. A. Lightfoot. 1996. Two additional Loci underlying Durable Field Resistance to Soybean Sudden Death Syndrome (SDS). Crop Science 36(5):(in press).

Heenan, D.P and L.C Campbell. 1980. Growth, yield components and seed composition of two soybean cultivars as affected by manganese supply. Aus. J. Agric. Res. 31: 471-476.

Hnetkovsky, N., S. J. C. Chang, T. W. Doubler, P. T. Gibson and D. A. Lightfoot. 1996. Genetic Mapping of Loci Underlying Field Resistance to Soybean Sudden Death Syndrome. (SDS). Crop Sci 36(2):(in press).

Ohki, K., F. C. Boswell, M. B. Parker, L. M Sherman, and D. O Wilson. 1987. Foliar manganese application to soybean. Agron. J. 72: 713-716.

Randall, G. W., E. E. Schulte, and R. B. Cory. 1975. Effect of soil and foliar applied manganese on the micronutrient content and yield of soybeans. Agron. J. 67: 502-507.

Rupe, J. C. W. E. Sabbe, R. T. Robbins and E. E. Gbur. 1994. Soil and plant factors associated with sudden death syndrome of soybean. J.Prod. Agric. 6:218-221.

Torto, G. A., V. Njiti and D. A. Lightfoot. 1996. Loci underlying resistance to sudden death syndrome and *Fusarium solani* in field and greenhouse assays do not correspond. Soybean Genetics Newsletter. (This issue)

V. Kilo

D. A. Lightfoot

Table 1: Molecular markers associated with Mn toxicity.

Marker	LG	n	Trait	R^2	*P*
$OF04_{1000}$	N	31	Mg1	0.28	0.002
			Mg2	0.14	0.033
			Mg3	0.20	0.011
			Mg5	0.23	0.006
			Mg7	0.25	0.004
			Mg8	0.21	0.009
			Mg4	0.23	0.004
$OC01_{650}$	N	29	Mg1	0.20	0.014
			Mg2	0.21	0.013
			Mg3	0.18	0.021
			Mg5	0.36	0.006
			Mg7	0.11	0.076
$OO04_{1075}$	N	32	Mg3	0.14	0.032
			Mg5	0.21	0.008
			Mg7	0.15	0.027
			Mg8	0.10	0.076
$OE04_{450}$	G	28	Mg1	0.26	0.006
$OE02_{1000}$	G	16	Mg1	0.35	0.016
			Mg2	0.34	0.02
			Mg7	0.30	0.03
			Mg8	0.30	0.03
$OG13_{490}$	G	32	Mg3	0.15	0.003
			Mg6	0.18	0.008
			Mg5	0.24	0.003
			Mg7	0.12	0.04
$OU15_{900}$	?	30	Mg6	0.36	0.005
			Mg2	0.36	0.02
$0C10_{600}$	?	32	Mg5	0.23	0.005
$0C11_{400}$	?	32	Mg6	0.26	0.002
			Mg5	0.25	0.004
			Mg7	0.13	0.04
			Mg8	0.16	0.023

SOUTHERN ILLINOIS UNIVERSITY
Department of Plant and Soil Science
Molecular Science Program
Carbondale, IL 62901.

Integration of SDS, SCN Race 3 and SCN Race 14 Resistance QTL with the Soybean Genome Map.

Two diseases of soybean (*Glycine max* (L.) Merr.), sudden death syndrome, caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans., type A, and soybean cyst nematode (SCN), caused by (*Heterodera glycines* Ichinohe) are becoming increasingly severe. Field resistance to both SDS and SCN race 3 is partly co-inherited (about 50%) when SCN resistance is derived from 'Peking' or PI88.788. Therefore, selection for partial resistance to both diseases is an economic control measure.

Conventional breeding methods have been used for selection of SDS resistant soybean cultivars. However, since SDS resistance is partial, polygenic and environmentally sensitive, breeding can be complicated due to the need for selection of many soybean lines and the effect of environmental conditions such as rainfall and soil fertility, maturity date and date of planting on SDS progression. Thus, for accurate and reliable field tests, multiple replications in several infested fields and collection of data at 3-4 day intervals is necessary. Therefore, breeding is time consuming, difficult and expensive.

Molecular biotechnology provides a genotype-based and thus more reliable method for identification and selection of SDS resistant soybean cultivars through the use of DNA markers. DNA markers in association with recombinant inbred lines can detect and map chromosomal regions containing SDS resistance quantitative trait loci. Random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), DNA amplification fingerprints (DAF) and microsatellite or simple sequence repeats (SSRs) are the different types of markers used for that purpose. However, genome maps produced by independent investigators in the same species are difficult to integrate except where SSR markers have been scored extensively. SSRs are the most informative markers, due to their high degree of allelic polymorphism, their specificity to a single locus and their broad distribution in the soybean genome. They can be useful markers to complement and integrate the RFLP and RAPD marker maps.

In this research, we demonstrate the use of molecular markers to map QTLs responsible for partial resistance to soybean SDS, SCN race 3 and SCN race 14 in two RIL populations derived from a cross between cultivars, Essex by Forrest and Pyramid by Douglas and the use of SSRs to integrate this map with other maps of the soybean genome.

Materials and Methods

Plant Material: The Essex by Forrest (ExF) F5 derived population of soybean recombinant inbred lines (RILs) is a typical "South by South" cross. Essex is SDS and SCN susceptible and Forrest is SDS and

SCN race 3 resistant. SCN resistance derives from Peking. The Pyramid by Douglas (PxD) F6 derived population of soybean recombinant inbred lines (RILs) is a typical "South by North" cross. Douglas is SDS and SCN susceptible and Pyramid is SDS, SCN race 3 and SCN race 14 resistant. SCN race 14 resistance derives from PI88.788.

SSR Protocol-Polymerase Chain Reaction (PCR): Amplifications were performed in a Perkin-Elmer 9600V thermal cycler after Akkaya et al., (1995). Primers were gifts from Dr. Cregan, USDA-ARS, Beltsville, MD or purchased from Research Genetics.

Results

Comparisons of markers: Five types of markers were evaluated and mapped in the ExF population (Table 1). SSR markers were among the most polymorphic of the markers tested. However, SSR polymorphism was relatively low compared to other soybean mapping populations (Akkaya et al., 1995) which reflects the limited genetic diversity typical of populations derived from adapted soybean germplasm in a current breeding program. The low frequency of RFLP polymorphism precludes map integration by RFLP alone as is possible in wider crosses (Shoemaker and Specht, 1995). RAPD, DAF and AFLP all combine high polymorphism frequency with facile methodologies. Any combination of the three markers would be suitable for rapid saturation of a linkage map in adapted germplasm although RAPD and DAF are not suitable for map integration.

Table 1. Differences in the polymorphism frequency between Essex and Forrest with RFLP, RAPD, DAF and SSR markers.

Molecular marker	Markers tested	Mappable bands	Percent Polymorphism
RFLP	243	58	24†
RAPD	600	108	18
DAF	10	6	60
SSR	50	17	34
AFLP	10	20	200

† Per five restriction enzyme digests

Map integration: SSR markers SATT79 and SCT28 allowed an SDS QTL from Essex (Hnetkovsky et al., 1996) to be anchored and oriented relative to a region of linkage group C2 (Shoemaker and Specht 1995) close to the telomere (Figure 1). However, the SSR is not close enough to allow detection of the equivalent QTL in other

populations. The marker $OO05_{450}$ maps to a region of the genome only sparsely populated with markers. Together with QTL linkage this may merit its conversion to an RFLP marker.

SSR markers SATT9 allowed an SDS QTL from Forrest (Hnetkovsky et al., 1996) to be excluded from one anchored region of linkage group N (Shoemaker and Specht 1995) (Figure 2). The assignment of this QTL to linkage group N will remain speculative until a linked SSR marker is mapped.

No SSR markers are currently commercially available that map to linkage group G where major loci for SCN race 3 (Webb et al., 1995), SCN race 1, SCN race 6 (Concibido et al., 1995) SCN race 14 and SDS resistance appear to map. Integration on this linkage group relies on RFLP markers and common QTL (Figure 3).

The SDS QTL on this linkage group is environmentally sensitive so that the Douglas allele is beneficial in three of six environments infested with SDS. The allelic Pyramid locus is most likely derived from PI88.788 since it is closely linked to SCN race 14 resistance. Breaking this linkage in a cross with a Peking source of resistance is of importance to improving SDS resistance since PI88.788 is a common source of SCN resistance in modern soybean germplasm.

References

Akkaya, M. S., R. C. Shoemaker, J. E. Specht, A. A. Bhagwat, P. B. Cregan. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci. 35:1439-1445.

Chang S. J. C., T. W. Doubler, V. Kilo, R. Suttner, J. Klein, M. E. Schmidt, P. T. Gibson and D. A. Lightfoot. 1996. Two additional loci underlying durable field resistance to soybean sudden death syndrome (SDS). Crop Science (submitted 10/23/95).

Concibido, V., Boutin, S., Denny R., Hautea R., Orf, J. Young, N. D. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (Heterodera glycine Ichinohe). Crop Sci. 34:240-246.

Concibido, V., Boutin, S., Denny R., Hautea R., Orf, J. and Young, N. D. 1995. The soybean cyst nematode resistance gene on linkage group G is common among sources of resistance. Soybean Genet. Newslett. 22:269-272.

Hnetkovsky, N., S. J. C. Chang, T. W. Doubler, P. T. Gibson and D. A. Lightfoot. 1996. Genetic Mapping of Loci Underlying Field Resistance to Soybean Sudden Death Syndrome (SDS). Crop Sci 36(2):(in press).

Shoemaker, R. C. and Specht, J. E. 1995. Integration of the soybean molecular and classical genetic linkage groups. Crop Sci. 35:436-446.

Webb, D. M., B. M. Baltazar, A. P. Rao-Arelli, J. Schupp, P. Keim, K. Clayton, A. R. Ferreira, T. Owens and W. D. Beavis. 1995. QTL affecting soybean cyst-nematode resistance. Theor. Appl. Genet. 91:574-581.

J. Abu-Thredeih
S. J. C. Chang
T. W. Doubler
D. A. Lightfoot

Figure 1: Comparison of marker positions between Clark x Harosoy (CxH) and Essex x Forrest (ExF) linkage group C2 that includes an SDS resistance QTL.

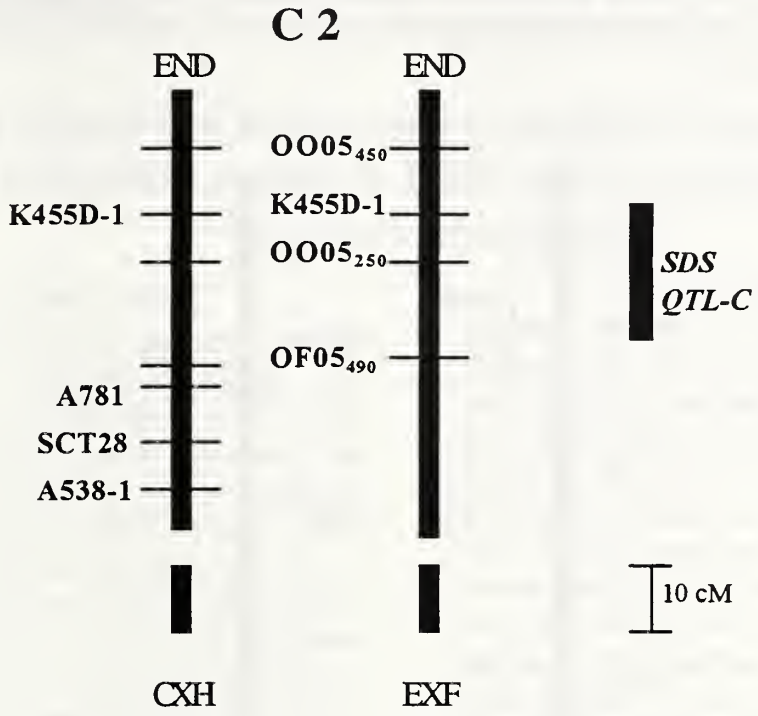


Figure 2: Comparison of marker positions between Clark x Harosoy (CxH) and two regions of Essex x Forrest (ExF) that might map to linkage group N including an SDS resistance QTL.

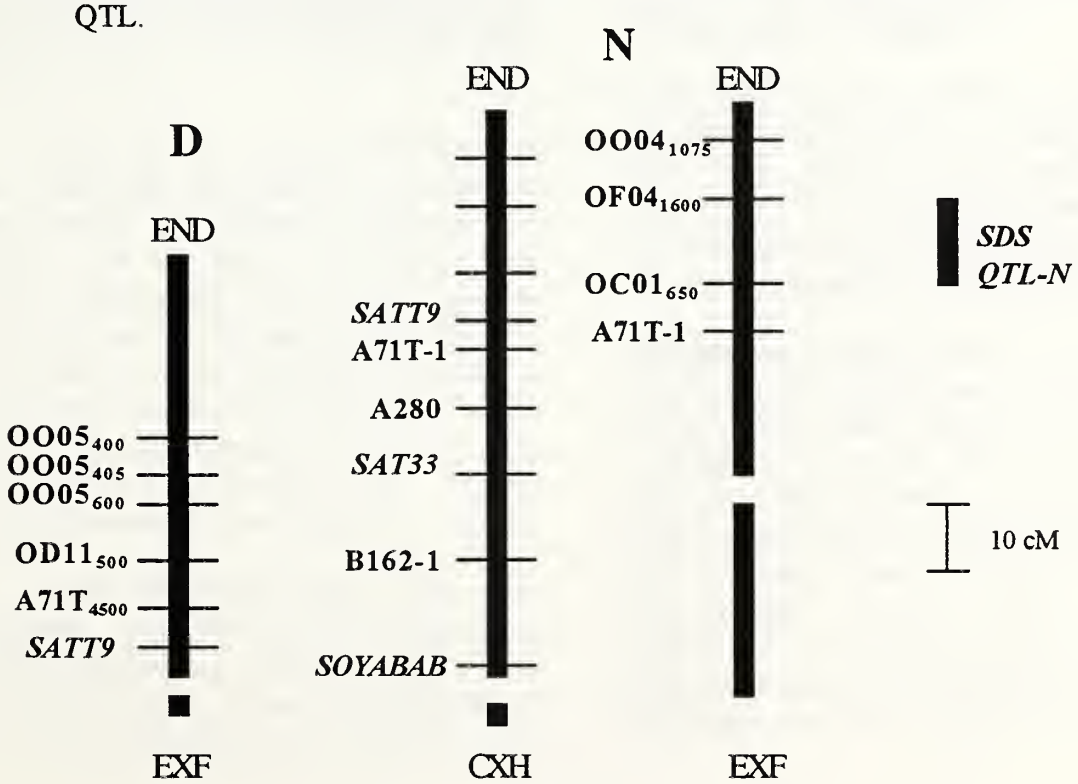
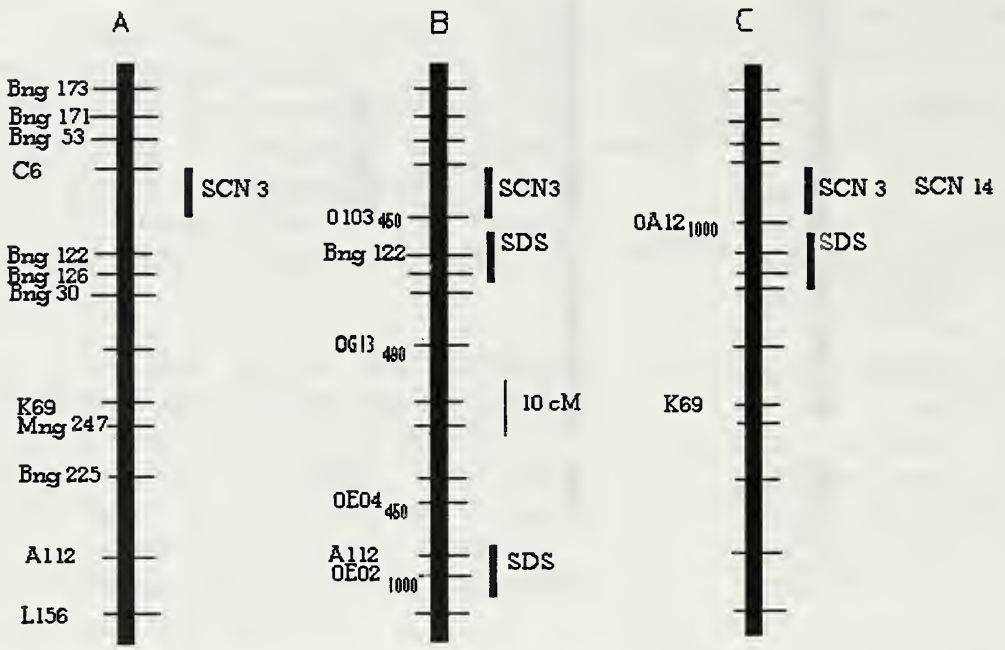


Figure 3: Comparison of marker positions between A, Evans x Peking B, Essex x Forrest and C, Pyramid x Douglas that might map to linkage group G including an SCN and two SDS resistance QTL.



SOUTHERN ILLINOIS UNIVERSITY
Department of Plant and Soil Science
Molecular Science Program
Carbondale, IL 62901

Loci Underlying Resistance to Sudden Death Syndrome and *Fusarium solani* in Field and Greenhouse Assays Do Not Correspond.

In 1971, a new fungal disease, soybean Sudden Death Syndrome (SDS) was documented in Arkansas (Roy et al., 1989; Rupe 1989). This disease has now been observed in most of the major producing areas in the United States. Sudden death syndrome in soybean (*Glycine max* (L.) is caused by *Fusarium solani* f.sp. *phaseoli* (O'Donell and Gray, 1995). Leaf symptoms include a mosaic-like coloration of the leaves followed by interveinal chlorosis and necrosis (Roy et al., 1989; Rupe, 1989). However, the fungus is restricted to the roots where infection causes rotting and discoloration in the tap root and a loss of lateral roots (Roy et al., 1989; Rupe, 1989; O'Donell and Gray, 1995).

Resistant or tolerant varieties are being sought to withstand the pressure of SDS. Field trials have been conducted over several years to select for resistant and tolerant varieties, but this is time consuming and expensive (Hnetkovsky et al., 1996). To offset some of these problems, screening in the greenhouse (Stephens et al., 1993) and marker assisted selection may be used as complementary techniques to develop improved varieties (Hnetkovsky et al., 1996).

Two greenhouse assays have been developed. The first assay is based on *F.solani* infested oat or sorghum seed placed close to the taproot of a seedling (Stephens et al., 1993). Plants are grown to maturity (until maximum contrast in the AUDPC for controls), DS is recorded every 48 h and plant weight is determined at the end of the experiment. The second assay is based on *F.solani* infested cornmeal and sand distributed throughout the soil (O'Donell and Gray, 1995). Plants are grown for only three weeks before data is recorded. DS and plant weights are recorded. Our goal was to compare the methods for accuracy in predicting SDS in the field using RILs from Essex x Forrest (ExF) which contrasted in their field SDS resistance (Hnetkovsky et al., 1996; Chang et al., 1996).

Materials and Methods

Fusarium solani strain ST90, maintained on 5X Bilays medium at 19 C, was transferred onto PDA plates at 28 C for inoculum preparation. The 40 ExF RILs used were the 20 most resistant to SDS in the field and the 20 most susceptible to SDS in the field.

Infested Seed Assay: Plants were inoculated employing the infested oat technique (Lim, 1991). Oats were soaked in tap water overnight, then excess water was strained away. The 150 ml portions were put into 250 ml Erlenmeyer flasks, capped, and autoclaved for 20 minutes. Oats were then cooled and three 1 cm^2 agar plugs from the colony borders of *F. solani* petri plates were aseptically added to the sterilized oats. The inoculated oats were incubated in the dark at

room temperature (24 C) for three weeks. Flasks were constantly shaken for uniform growth. Plants were grown in a soil medium containing a 1:1 (v/v) mixture of sterilized sand and sterilized soil taken from Ridgway. Inoculation of the plants was done at V2-V3 stage of growth by placing five infested oats next to the taproot below the soil surface. Plants were then irrigated to seal the soil, and soil was kept saturated to ensure the pathogenicity of the fungus.

Cornmeal Sand Assay: The cornmeal method of inoculation was used with a few modifications. Two 1 cm X 1 cm square pieces of the infested agar was transferred into a 50 ml volume of cornmeal and silica mix (1:1) and moistened with sterile water. This was kept in an incubator for 14 days. At the same time seeds were sown on steamed sand . After 14 days, the inoculum was mixed with steamed sand/soil (1:1 v/v) in a 1: 40 proportion. Styrofoam cups were filled and 14 day old seedlings were transferred into the filled cups. These were set in water filled basins with enough water to keep them moist. The experimental design was a 2- factor randomized complete block with treatment as a split on genotype. After 21 days, disease severity based on a scale of 1-9 (1 = plants with no leaf chlorosis and 9 = dead plant). was scored. Other trait data scored were root weight, shoot weight and plant height. Data analysis used a one-way analysis of variance (ANOVA).

Results and Discussion

Greenhouse traits: There was a high significant difference between treatments for EXF and the RILs (Table 1). This significant dry root weight and shoot weight confirm the pathogenicity of the strain of *Fusarium solani* used and the effectiveness of the assays as a whole. As seen on the same table, there was significance among the lines for all the traits measured except disease severity. The frequency distribution of disease severity for the EXF RILs was normal ($P > 0.16$) not bimodal. The field scores and greenhouse scores were not significantly correlated for either assay.

Table 1. Probabilities of F for variance among lines and treatments

Source	df	DS	RWT	DSHWT	HT
LINE	41	ns	0.001	0.03	0.05
TREAT	1	0.001	0.001	0.001	0.001

DS- Disease Severity. RWT- Dry root weight. SHWT- Dry Shoot Weight. Ht- Height

Marker associations: Five markers were associated with the traits measured for the forty lines tested in the cornmeal sand assay (Table 2). $OO19_{400}$ was strongly associated with both plant height and shoot dry weight. The beneficial allele was derived from Essex. The other markers were only marginally significant ($P > 0.01$) and none corresponded to the QTL underlying field SDS resistance (Hnetkovsky et al., 1996; Chang et al., 1996).

Further analysis using the extreme twenty lines of EXF (10 best and 10 worst for field SDS score) showed three markers previously identified as being significantly associated with field disease resistance to be associated with some traits in the greenhouse. The two greenhouse assays detected distinct loci as associated with SDS. However, for $OE02_{1000}$ and $OC01_{650}$ the beneficial allele in the greenhouse was opposite to that in the field.

Table 2. Marker association with traits in the cornmeal sand assay using the extreme 40 ExF lines for field SDS.

MARKER	LG	TRAIT	P	R_2	ALLELIC	MEANS
					E	F
$OO19_{400}$	?	HT	0.0001	0.348	68.2	88.9
$OO19_{400}$	?	SHWT	0.005	0.203	48.8	61.2
$OE01_{450}$	?	RWT	0.034	0.188	38.7	58.1
$OC11_{300}$	N	SHWT	0.034	0.125	59.3	49.6
A063	C1	DS	0.028	0.429	5.0	3.0

DS- Disease Severity. RWT- Dry root weight. SHWT- Dry Shoot Weight. HT- Height

Table 3. Markers showing significant association with field disease parameters as well as greenhouse traits.

MARKER	LG	P(field)	TRAIT	P	R^2	ALLELIC	MEANS
						E	F
a, Cornmeal Assay							
$OR10_{380}$	Anon1	0.036	DS	0.010	0.440	3.4	5.1
$OR10_{400}$	Anon1	0.036	DS	0.010	0.440	3.4	5.1
$OE02_{1000}$	G	0.003	RWT	0.006	0.809	90.4	39.0
$OE02_{1000}$	G	0.003	SHWT	0.017	0.707	70.3	48.0
b, Infested Seed Assay							
$OC01_{650}$	N	0.001	SHWT	0.004	0.44	109.0	70.6

DS- Disease Severity. RWT- Dry root weight. SHWT- Dry Shoot Weight. HT- Height

None of the markers that showed the expected association between *F.solani* resistance in the greenhouse and SDS resistance in the field. The use of the most extreme genotypes does not increase the accuracy of detection of the field QTL. It is possible the greenhouse assays will detect SDS resistance QTL for which the ExF population do not contrast or for traits that are not measured in field assays (root weight). However, we conclude that further refinement of the greenhouse assays are necessary before the assays can make any meaningful contribution to the improvement of soybean SDS resistance. We encourage researchers developing greenhouse assays for SDS to use the immortal population of ExF RILs and molecular map as material for validating "improved" assays of SDS. Until an effective greenhouse assay is developed and proven effective (see Kilo and Lightfoot, 1996), soybean improvement by field evaluation and marker assisted selection will be pursued.

References

Chang S. J. C., T. W. Doubler, V. Kilo, R. Suttner, J. Klein, M. E. Schmidt, P. T. Gibson and D. A. Lightfoot. 1996. Two additional Loci underlying Durable Field Resistance to Soybean Sudden Death Syndrome (SDS). Crop Science 36(5):(in press).

Hnetkovsky, N., S. J. C. Chang, T. W. Doubler, P. T. Gibson and D. A. Lightfoot. 1996. Genetic Mapping of Loci Underlying Field Resistance to Soybean Sudden Death Syndrome (SDS). Crop Sci 36(2):(in press).

Kilo, V. and D. A. Lightfoot. 1996. Genetic analysis of resistance to manganese toxicity in soybean. Soybean Genetic Newsletter. (this issue).

O'Donell K. and L. E. Gray 1995. Phylogenetic Relationships of the Soybean Sudden Death Syndrome Pathogen *Fusarium solani* f.sp. *phaseoli* Inferred from rDNA Sequence Data and PCR Primers for Its Identification. Molecular Plant Microbe Interactions . 8:709-716.

Roy, K. W., G. W. Lawrence, H. H. Hodges, K. S. Mclean, and J. F. Killebrew. 1989 Sudden Death of Soybean: *Fusarium solani* as Incitant and Relation of *Heterodera glycines* to Disease Severity. Phytopathology. 79:191-197

Rupe, J. C. 1989. Frequency and Pathogenicity of *Fusarium solani* Recovered from Soybeans with Sudden Death Syndrome. Plant Disease 73:581-584.

Stephens, P. A., C. D. Nickell, C. K. Moots and S. M. Lim. 1993. Relationship between Field and Greenhouse Reaction to *Fusarium solani* in Soybean. Crop Sci. 33:929-930.

G. A. TORTO*
V. NJITI
D. A. LIGHTFOOT

LOUISIANA STATE UNIVERSITY AGRICULTURAL CENTER
Dean Lee Research Station
8105 East Campus Avenue
Alexandria, LA 70312

Investigation of Transferring the BAR Gene into Soybean Via the Pollen-Tube Pathway

Traditional plant breeding has utilized cross pollination procedures to develop genetic diversity from which superior genotypes could hopefully be selected. With the advent of new biotechnology tools, genes from one species are now transferred to others in the laboratory. Frequently, foreign genes are inserted into soybean DNA through the use of a "gene gun" which propels gold particles covered with a gene construct into soybean protoplasm. Tissue culture is required to obtain explants from the treated protoplasm. The technique studied here, first learned of by the principle investigator in China, reportedly transfers genes by direct application of solutions containing DNA or gene constructs through pollen tubes. Successful use of this technique has apparent major advantages, including immediate transfer of foreign genes into established varieties, an end product being a whole seed which may easily be propagated, and elimination of costly time and equipment required for gene guns and subsequent tissue culture. Numerous Chinese scientists have apparently reported success in using this technique, based on variability recorded in progeny. Wu and Luo (1989) reported up to 20% efficiency using this method to transfer DNA into rice in a U.S. laboratory.

Materials and Methods

Solutions containing the BAR gene for Glufosinate ('Ignite' or 'Liberty') resistance, coupled to an actin promoter, were applied to severed styles of flowers from field grown 'BS 723 and 'Vernal' soybean varieties in the summer of 1995. A numerical rating on a 1 to 5 scale (1=corolla just beginning to extend from the calyx, 5=a fully opened flower) was used to describe the stage of maturity of the flower when treated. The time between pollination and fertilization of the embryo in soybeans is generally from 8 to 10 hours. The objective was to apply solutions containing the BAR gene behind gametes going down the pollen tubes, but before fertilization. Styles were severed with a scalpel and usually about 2 microliter of solution was applied with a gas chromatograph needle to the severed surface. Fifty seed from treated flowers were harvested at maturity and later planted in the greenhouse. Five seed of each variety were also planted as checks. Plants were sprayed with 'Liberty' (Glufosinate) herbicide on 21 December at a targeted rate of 750 g ai per hectare. Solution was broadcast at 15 GPA using 30 PSI with 8004 tips.

Results and Discussion

All soybean plants were decisively killed by the application of Liberty herbicide, indicating that the gene was not successfully incorporated into the DNA to provide resistance. In addition, use of this method in a similar study with cotton resulted in the death of 226 cotton seedlings without any showing resistance to Liberty.

Conclusion

Numerous Chinese publications have reported apparent success in transferring DNA into cotton, rice, and soybean using pollen-tube pathways. Papers have reported applying DNA solutions to the stigma or injecting DNA solutions directly into the ovary. Wu and Luo (1989) reported success with this technique in rice. At the present, the authors have not been successful in using this technique. The idea of making genes physically present within ovules during fertilization is appealing and perhaps more refinement of the technique or more attempts are needed to obtain success. Experiments with this method are planned for 1996.

References

Wu, R. and Z. Luo. 1989. A simple method for the transformation of rice via the pollen-tube pathway. Plant Molecular Biology Reporter 7:69-77.

Steven Moore
Timothy Croughan
Gerald Myers
Roy Vidrine

MICHIGAN STATE UNIVERSITY[1]
Department of Crop and Soil Sciences
East Lansing, MI 48824

CLEMSON UNIVERSITY[2]
Department of Agronomy and
Department of Biological Sciences
Clemson, SC 29631

UNIVERSITY OF MISSOURI[3]
Plant Science Unit
Columbia, MO 65211

IOWA STATE UNIVERSITY[4]
Department of Agronomy
Ames, IA 50011

The Genetic Relationship among Plant Introductions with Resistance to Soybean Cyst Nematodes

Soybean cultivars in the U.S. have resistance to soybean cyst nematodes (SCN) (*Heterodera glycines* Ichinohe) derived mostly from the plant introductions (PIs) PI 88788 and 'Peking' (Rao Arelli, 1994). Breeders need to increase the genetic diversity of SCN resistance genes in cultivars. The use of resistant cultivars will cause nematode race shifts that will lead to their genes no longer providing resistance.

There are many PIs in the USDA Soybean Collection that have been identified as resistant to SCN (Ross and Brim, 1957; Epps and Hartwig, 1972; Anand and Gallo, 1984; Young, 1990) and some could be used in breeding programs to broaden the diversity of resistance genes. The objectives of this study were to describe the genetic relationship among SCN resistant PIs and determine the resistance response of these PIs to *H. glycines* races 1, 2, 3, 5, and 14 in controlled conditions in the greenhouse.

Materials and Methods

SCN races: Near-homogeneous populations of *H. glycines* races were produced according Anand and Rao Arelli (1989). Race 1 was maintained on 'Essex', race 2 on 'Pickett-71', race 3 on Essex, race 5 on PI 88788, and race 14 on a mixture of 'Forrest' and PI 90763. The races were tested by inoculating soybean differentials (Riggs and Schmitt, 1987) prior to their use in this experiment.

A set of 38 soybean PIs, the four host differentials (Peking, PI 90763, PI 88788 and Pickett-71), and Essex, the susceptible control, were tested for resistance to five *H. glycines* races according to procedures by Rao Arelli (1994). There were 40, 1-plant replicates for each soybean genotype. Approximately 30 days after inoculation, plant roots were individually washed and the number of females and cysts were counted under a stereomicroscope. An index of parasitism (IP) was calculated for each accession according to Golden et al. (1970). The IP was calculated by dividing the average number of cysts and females counted on the accessions by the average number of cysts and females counted on Essex multiplied by 100. Ratings of resistant (IP = 0-9%), moderately resistant (IP = 10-30%), moderately susceptible (IP = 31-60%) and susceptible (IP $\geq$ 60%) were used to classify the accessions (Schmitt and Shannon, 1992).

Genetic Analysis: The group of resistant PIs, the differentials and 12 elite soybean cultivars (Table 1) were evaluated with RFLP markers according to protocols described by Diers and Osborn (1994) or Skorupska et al. (1993). The DNA clones used as probes for RFLP analysis were developed by Dr. Randy Shoemaker, Iowa State University-USDA ARS, and were obtained from Biogenetic Services, Inc. (Brookings, SD). Each polymorphic RFLP fragment was scored as present or absent and a genetic similarity matrix of the soybean genotypes was calculated using the simple matching coefficient. A cluster analysis was done using the similarity matrix and the unweighted pair group method, arithmetic average (UPGMA). These analyses were done with the computer program NTSYS (Exeter Software, Setauket, New York).

Results and Discussion

The PIs were evaluated for resistance to races 1, 2, 3, 5, and 14. Thirteen of the PIs were classified as resistant to race 1, 5 resistant to race 2, 24 resistant to race 3, 17 resistant to race 5 and 8 resistant to race 14 (Table 1). The high proportion of PIs with resistance to race 3 may be at least partially the result of many of the PIs being initially identified in a screening of the germplasm collection with race 3. PI 437654 and PI 438489B were the only PIs found to be resistant to all five races of *H. glycines* in this study.

The SCN resistant PIs and 12 elite cultivars were screened with 201 DNA clones. One hundred and thirty nine clones were polymorphic revealing 316 polymorphic fragments. The polymorphic fragments were used in a cluster analysis to group the soybean genotypes based on their genetic similarities. The cluster analysis placed the PIs into several groups and with only two exceptions, uniquely grouped the northern cultivars (Maturity Groups I-III) and the southern cultivars (Maturity Groups IV-VII) (Figure 1). Two PIs, PI 398682 and Patoka, grouped with the southern cultivars.

Many PIs that were closely grouped had identical or similar resistance reactions (Figure 1). For example, PI 404198B and PI 303652 were closely grouped and had identical resistance reactions. There were also large groups of PIs with common resistance reactions. For example, the group of PIs on the upper part of the dendrogram from PI 398682 to PI 91138 all have greater susceptibility to SCN races than the PIs on the lower part of the dendrogram. The PIs in the lower portion of the dendrogram are divided into two major groups, an upper group that extends from Ilsoy to PI 437655 and a lower group that extends from Peking to PI 438489B. These two groups differ for resistance to race 2 with the upper group mostly susceptible or moderately susceptible and the lower group mostly resistant or moderately resistant. The cluster analysis grouped genotypes that share marker alleles that were either identical by descent or alike in state. It is therefore likely that these groups of genotypes will also have resistance genes in common which may explain why they have similar resistance reactions.

An objective of this study was to identify PIs resistant to *H. glycines* that are distantly related to resistance sources presently being used by breeders. These PIs would have a high probability of providing new resistance genes that will contribute to the broadening of the genetic bases of SCN resistance available in cultivars. The group of PIs most distant from the previously used sources is in the upper portion of the dendrogram. Although these PIs have

moderate resistance only to race 3, they need to be evaluated as potential sources of resistance to this race. Another PI that should be evaluated as a resistance source is PI 438489B. This PI is resistant to all five races and although in a cluster with Peking, it is not closely grouped with this presently used resistance source.

References

Anand, S. C., and K. M. Gallo. 1984. Identification of additional soybean germplasm with resistance to race 3 of the soybean cyst nematode. Plant Dis. 68:593-595.

Anand, S. C., and A. P. Rao Arelli. 1989 Genetic analyses of soybean genotypes resistant to soybean cyst nematode race 5. Crop Sci. 29:1181-1184.

Diers, B. W. and T. C. Osborn. 1994. Genetic diversity of oilseed *Brassica napus* germplasm based on restriction fragment length polymorphisms. Theor. Appl. Genet. 88:662-668.

Epps, J. M., and E. E. Hartwig. 1972. Reaction of soybean varieties and strains to race 4 of the soybean cyst nematode. J. Nematol. 4:222 (Abstract).

Golden, A. M., J. M. Epps, R. D. Riggs, L. A. Duclos, J. A. Fox, and R. L. Bernard. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Disease Reporter 54:544-546.

Rao Arelli, A. P. 1994. Inheritance of resistance to *Hetertodera glycines* race 3 in soybean accessions. Plant Dis. 78:896-900.

Riggs, R. D., and D. P. Schmitt. 1987. Complete characterization of the race scheme for *Heterodera glycines*. J. Nematol. 20:392-395.

Ross, J. P., and C. A. Brim. 1957. Resistance of soybeans to the soybean cyst nematode as identified by a double-row method. Plant Dis. Rep. 41:923-924.

Schmitt, D. P., and G. Shannon. 1992. Differentiating soybean responses to *Heterodera glycines* races. Crop Sci. 32:275-277.

Skorupska, H. T., R. C. Shoemaker, A. Warner, E. R. Shipe, and W. C. Bridges. 1993. Restriction fragment length polymorphism in soybean germplasm of the southern USA. Crop Sci. 33:1169-1176.

Young, L. D. 1990. Soybean germplasm evaluated for resistance to races 3, 5, and 14 of soybean cyst nematode. Crop Sci. 30:735-736.

B. W. Diers[1]
H. T. Skorupska[2]
A. P. Rao-Arelli[3]
S. R. Cianzio[4]

Table 1. Plant introductions and cultivars, their maturity groups, year of introduction, origin and resistance reactions.

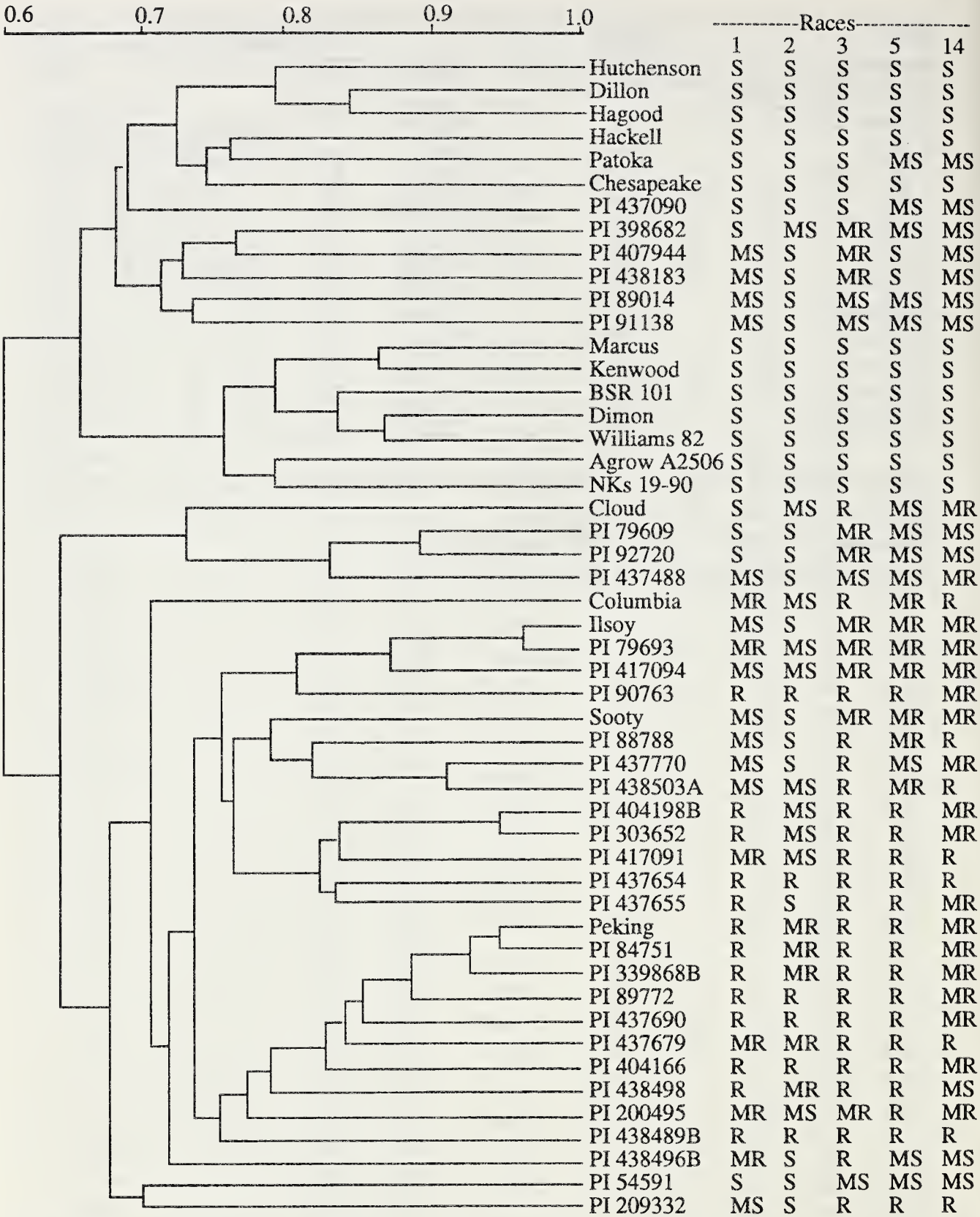
Strain Designation	Maturity Group	Year of Introd.	Origin	Race 1	Race 2	Race 3	Race 5	Race 14
Cyst Nematode Resistant PIs								
PI 16790 Sel. (Cloud)	III	1905	China	MS†	S	R	MS	MR
PI 22897 (Columbia)	III	1908	China	MR	MS	R	MR	R
PI 6387 Sel. (Ilsoy)	III	1901	Korea	MR	MS	MR	MR	MR
PI 70218-2-19-3 (Patoka)	IV	1926	China	S	S	S	S	MS
PI 17852-B Sel. (Peking)	IV	1906	China	R	MR	R	R	MR
Sooty	IV			MS	S	MR	MR	MR
PI 54591	III	1921	China	S	S	MS	MS	MS
PI 79609	II	1928	China	S	S	MR	MS	MS
PI 79693	III	1929	China	MR	MS	MR	MR	MR
PI 84751	IV	1930	China	R	MR	R	R	MR
PI 88788	III	1930	China	MS	S	R	MS	R
PI 89014	II	1930	China	MS	S	MS	MS	MS
PI 89772	IV	1930	China	R	R	R	R	MR
PI 90763	IV	1930	China	R	R	R	R	MR
PI 91138	II	1930	China	MS	S	MS	MS	MS
PI 92720	III	1931	China	S	S	MR	MS	MS
PI 200495	IV	1952	Japan	MR	MS	MR	R	MR
PI 209332	IV	1953	Japan	MS	S	R	R	R
PI 303652	V	1965	Australia‡	R	MS	R	R	MR
PI 339868B	IV	1968	Korea	R	MR	R	R	MR
PI 398682	IV	1974	Korea	S	MS	MR	MS	MS
PI 404166	III	1975	Russia‡	R	R	R	R	MR
PI 404198B	IV	1975	Russia‡	R	MS	R	R	MR
PI 407944	V	1976	Korea	MS	S	MR	S	MS
PI 417091	II	1977	Japan	MR	MS	R	R	R
PI 417094	III	1977	Japan‡	MS	MS	MR	MR	MR
PI 437090	O	1980	Russia	S	S	S	MS	MS
PI 437488	II	1980	Russia‡	MS	S	MS	MS	MR
PI 437654	III	1980	Russia‡	R	R	R	R	R
PI 437655	III	1980	Russia‡	R	S	R	R	MR
PI 437679	IV	1980	Russia‡	MR	MR	R	R	R
PI 437690	III	1980	Russia‡	R	R	R	R	MR
PI 437770	III	1980	Russia‡	MS	S	R	MS	MR
PI 438183	II	1980	Russia‡	MS	S	MR	S	MS
PI 438489B	IV	1980	Russia§	R	R	R	R	R
PI 438496B	III	1980	Russia§	MR	S	R	MS	MS
PI 438498	IV	1980	Russia§	R	MR	R	R	MS
PI 438503A	II	1980	Russia§	MS	MS	R	MR	R

Strain Designation	Maturity Group	Year of Introd.	Origin	Race 1	Race 2	Race 3	Race 5	Race 14
Susceptible Cultivars								
Asgrow 2506	II		Asgrow	S	S	S	S	S
BSR101	I		ISU	S	S	MS	S	S
Chesapeake	IV		Virginia	S	S	S	S	S
Dillon	VI		Clemson	S	S	S	S	S
Dimon	II		MSU	S	S	S	S	S
Hagood	VII		Clemson	S	S	S	S	S
Haskell	VII		Georgia	S	S	S	S	S
Hutchenson	V		Virginia	S	S	S	S	S
Kenwood	II		ISU	S	S	S	S	S
Marcus	I		ISU	S	S	S	S	S
NKs19-90	I		N. King	S	S	S	S	S
Williams 82	III		Illinois	S	S	S	S	S

† S=Susceptible (IP ≥ 60%), R= Resistant (IP = 0-9%), MR=Moderately resistant (IP = 10-30%), MS=Moderately susceptible (IP = 31-60%).

‡ Originally collected in China (Bernard et al., 1989).

§ Provided to Russia from the U.S (Bernard et al., 1989). .



	Races				
	1	2	3	5	14
Hutchenson	S	S	S	S	S
Dillon	S	S	S	S	S
Hagood	S	S	S	S	S
Hackell	S	S	S	S	S
Patoka	S	S	S	MS	MS
Chesapeake	S	S	S	S	S
PI 437090	S	S	S	MS	MS
PI 398682	S	MS	MR	MS	MS
PI 407944	MS	S	MR	S	MS
PI 438183	MS	S	MR	S	MS
PI 89014	MS	S	MS	MS	MS
PI 91138	MS	S	MS	MS	MS
Marcus	S	S	S	S	S
Kenwood	S	S	S	S	S
BSR 101	S	S	S	S	S
Dimon	S	S	S	S	S
Williams 82	S	S	S	S	S
Agrow A2506	S	S	S	S	S
NKs 19-90	S	S	S	S	S
Cloud	S	MS	R	MS	MR
PI 79609	S	S	MR	MS	MS
PI 92720	S	S	MR	MS	MS
PI 437488	MS	S	MS	MS	MR
Columbia	MR	MS	R	MR	R
Ilsoy	MS	S	MR	MR	MR
PI 79693	MR	MS	MR	MR	MR
PI 417094	MS	MS	MR	MR	MR
PI 90763	R	R	R	R	MR
Sooty	MS	S	MR	MR	MR
PI 88788	MS	S	R	MR	R
PI 437770	MS	S	R	MS	MR
PI 438503A	MS	MS	R	MR	R
PI 404198B	R	MS	R	R	MR
PI 303652	R	MS	R	R	MR
PI 417091	MR	MS	R	R	R
PI 437654	R	R	R	R	R
PI 437655	R	S	R	R	MR
Peking	R	MR	R	R	MR
PI 84751	R	MR	R	R	MR
PI 339868B	R	MR	R	R	MR
PI 89772	R	R	R	R	MR
PI 437690	R	R	R	R	MR
PI 437679	MR	MR	R	R	R
PI 404166	R	R	R	R	MR
PI 438498	R	MR	R	R	MS
PI 200495	MR	MS	MR	R	MR
PI 438489B	R	R	R	R	R
PI 438496B	MR	S	R	MS	MS
PI 54591	S	S	MS	MS	MS
PI 209332	MS	S	R	R	R

Figure1. Dendrogram of soybean Pls and cultivars obtained from an analysis of 139 polymorphic RFLP probes. Included are the resistance reactions of the genotypes to five *H. glycines* races.

UNIVERSITY OF MINNESOTA
Department of Agronomy and Plant Genetics [1]
Department of Plant Pathology [2]
St. Paul, Minnesota 55108

Genetic Mapping of a Yield Depression Locus Near a Major Gene for Soybean Cyst Nematode Resistance

Introduction

The use of cultivars with resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN), is currently the most effective method for controlling this destructive pathogen. However, cultivars selected for resistance to SCN typically have lower yields than susceptible cultivars when grown in the absence of SCN pressure. One reason for the decreased yield may be linkage drag between SCN resistance and a yield depression locus. Molecular markers linked to these traits might be useful for tracing both the SCN resistance and the yield depression locus. Thus, marker-assisted selection can accelerate the breeding process and may be particularly useful in breaking the linkage between the two loci.

Materials and Methods

Plant Materials: Two populations were used in this research. The first is an RIL (recombinant inbred line) population consisting of 98 $F_{5:7}$ lines developed from the cross 'Evans' x PI 209332. The second is a population derived from line 44. Line 44 is an F_3 line selection from the cross M83-15 x M85-1430 (with PI 209332-derived SCN resistance; Concibido *et al.*, 1994), which was heterozygous and thus segregated in the region of the SCN resistance and yield depression locus. Individual plants from line 44 were crossed to Evans. Two F_2 seeds from every F_1 plant were planted and advanced to the F_5 generation using single seed descent. Between 15 and 20 lines from this line 44-derived population were tested each year.

The line 44-derived population ($F_{5:6}$) was yield-tested in St. Paul, MN in 1994. In 1995, both populations were grown in Waseca (non-SCN-infected) and New Richland (SCN-infected), MN with two replications at each site. Due to flooding at New Richland, the second replication was dropped and the first replication only was used to confirm putative quantitative trait loci (QTL) uncovered with data averaged across the replications from Waseca, unless otherwise noted.

DNA Markers: Plants in the F_5 RIL and $F_{5:7}$ line 44-derived populations were genotyped as described by Concibido *et al.* (1994). Informative RFLP markers genotyped in the line 44-derived population were all on linkage group G. Development of the RFLP linkage map has been previously described (Concibido *et al.*, 1994, Keim *et al.*, 1990).

QTL Analysis: Data for seed yield, plant height, maturity, lodging, and seed protein and oil content were analyzed using Statview-II regression analysis and single-factor ANOVA. Linkage between a marker and a QTL was considered significant at $p \leq 0.01$.

Results and Discussion

Previous research has demonstrated that a major partial SCN resistance gene in both populations is located between C6 and Bng122 on linkage group 'G' in PI 209332 (Figure 1; Concibido *et al.*, 1994, 1996).

Yield. QTL linear regression data are summarized in Table 1. In the RIL population, a putative locus for yield depression was identified in the same genomic interval (between Bng173 and C6) as previously identified for SCN resistance (Figure 1). In the line 44-derived population, by contrast, a putative locus for yield depression was uncovered on linkage group 'G' in the interval between Bng122 and Mng247 (Figure 1). This interval is approximately 10 cM away from that identified in the RIL population described above and is located on the opposite side of the SCN resistance locus.

It is possible that the same locus was uncovered in both populations and that the discrepancy was due to the small population sizes involved, especially with the line 44-derived population. Also, two different sources of PI 209332 were used in developing the populations, so it is possible that two distinct yield loci exist. Knowing the precise location of the yield depression locus (loci) has important implications for the possibility of breaking its linkage with the SCN resistance allele. Future yield trials with the two populations, as well as mapping in additional PI 209332 populations should further define the genetic basis of yield depression.

The Evans x PI 209332 RIL population grown in Waseca exhibited a second yield depression locus near A235 on linkage group K. By contrast, the PI 209332 allele at A520 on linkage group B was associated with increased yield in the New Richland location though this marker was not significant at Waseca. The flooded or SCN-infested conditions at New Richland may have caused this locus, associated with height, maturity, and lodging (see below), to have a pleiotropic effect on yield.

Other Agronomic Traits. PI 209332 alleles at A520 and A89 on linkage group B showed significant association with later maturity and increased height at both sites, as well as increased yield in New Richland. Decreased protein content and increased lodging were also associated with the A89 allele. At New Richland, A520 was also associated with lodging ($p=0.001$, slope=0.4).

Decreases in both seed protein and oil content were strongly linked to markers K400, A85, and A486 on linkage group A. This genomic region has been previously shown to be associated with SCN resistance (Concibido *et al.*, 1994, Webb *et al.*, 1995).

Finally, K69 and Mng247, near the SCN resistance region on 'G' (Figure 1), were linked to increased oil in the line 44-derived population. A86 on linkage group E was associated with later maturity and increased height.

Table 1. Summary of QTL data

Trait	Markers	LG[a]	p value	Slope[b]	R^{2c}	Pop
Yield	A520[d]	B	0.0029	3.1	0.12	RIL
bu/acre	Bng173/C6	G	0.0069	-2.2	0.08	RIL
	Bng122/K69/Mng247	G	0.0071	-4.7	0.44	L44[e]
	A235	K	0.0048	-2.3	0.09	RIL
Height	A89/A520	B	0.0039	2.3	0.09	RIL
inches	A86/A386	E	0.0013	2.6	0.12	RIL
Lodging[f]	A89	B	0.0088	0.2	0.08	RIL
Maturity	A89/A520	B	0.0001	4.6	0.20	RIL
Days after 8/31	A86	E	0.0040	3.2	0.09	RIL
Protein	K400/A85/A486	A	0.0001	-1.5	0.43	RIL
%	A89	B	0.0074	-0.7	0.09	RIL
Oil	K400/A85/A486	A	0.0001	-1.3	0.49	RIL
%	K69/Mng247	G	0.0166	0.4	0.37	L44

[a] Linkage Group

[b] Refers to the positive or negative effect of the PI 209332 allele on the trait.

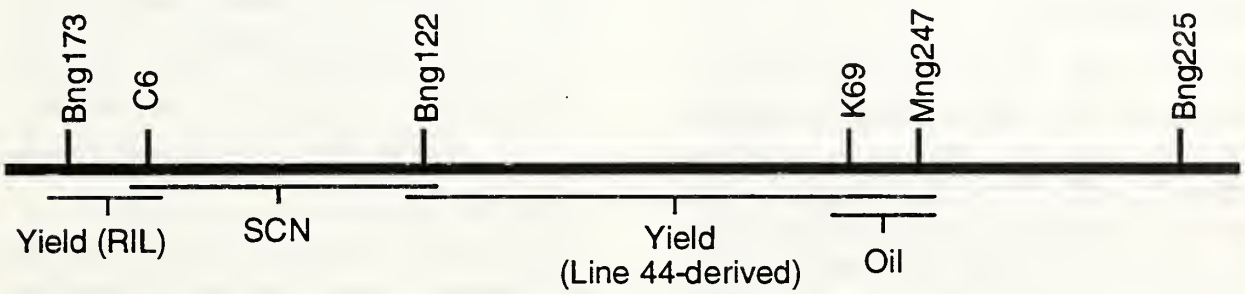
[c] Proportion of the variation explained by the difference between homozygous Evans and PI 209332 genotypes.

[d] Data are from New Richland Rep 1. It is not significant at Waseca.

[e] Line-44 derived population.

[f] Scored 1 (erect) to 5 (completely lodged).

Figure 1. Linkage Group 'G' near the SCN resistance locus



Implications for Marker-Assisted Selection.

The location of the PI 209332 allele(s) for yield depression must be narrowed down further to determine the precise location(s) and to confirm that it is distinct from the SCN resistance locus. If so marker-assisted selection should be effective at breaking the linkage between the two loci. Further yield trials involving the two populations in this study, as well as larger mapping populations currently under development, should help clarify the site of this yield depression locus (loci).

The linkage of markers A520/A89 and A86 to several agronomic traits makes multiple trait selection based on DNA markers possible. However, the linkage also complicates marker-assisted selection. The yield depression locus associated with A520 identified in New Richland emphasizes the importance of environmental conditions in controlling pleiotropic effects of loci. Also, a marker may be linked to both positive and negative traits. It is not known whether there is a gene cluster in these genomic regions, with each locus controlling a different trait, or if there are one or more genes with pleiotropic effects.

Acknowledgments

This research was supported by the Minnesota Crop Improvement Association (MCIA) and the Minnesota Soybean Research and Promotion Council. This article is published in the series of the Minnesota Agricultural Experiment Station. Results from this article may be cited without contacting the authors for permission, as long as a standard citation to the source of the work is included.

References

Concibido, V. C., R. L. Denny, S. R. Boutin, R. Hautea, J. H. Orf, N.D. Young. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Sci. 34 (1): 240-246.

Concibido, V. C., R. L. Denny, D. A. Lange, J. H. Orf, N. D. Young. 1996. RFLP mapping and marker-assisted selection of soybean cyst nematode resistance in PI 209332. Crop Sci. (Submitted for publication).

Keim, P., B. W. Diers, T. C. Olson, R. C. Shoemaker. 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126 (3): 735-742.

Webb, D. E., B. M. Baltazar, A. P. Rao-Arelli, J. Schupp, K. Clayton, P. Keim, W.D. Beavis. 1995. Genetic mapping of soybean cyst nematode race 3 resistance loci in the soybean PI 437.654. Theor. Appl. Genet. 91:574-581.

Joann Mudge[1]
Vergel Concibido[2]
Roxanne Denny[2]
Nevin Young[2]
James Orf[1]

UNIVERSITY OF MINNESOTA
Department of Plant Pathology [1]
Department of Agronomy and Plant Genetics [2]
St. Paul, Minnesota 55108

Marker-Assisted Selection for Soybean Cyst Nematode Resistance

Introduction

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is the most serious disease of soybeans (*Glycine max* L. Merr.) today. Farmers use a variety of strategies for managing SCN, especially crop rotation and resistant varieties. However, breeding resistant varieties is time-consuming and difficult. Several genes are involved, there are many resistant parents used in breeding, and the nematode exists as complex populations of different races. These difficulties have a serious side-effect — resistant varieties generally show lower yield in the absence of nematodes.

The breeding of SCN resistant varieties should be simpler and faster with the aid of marker-assisted selection (MAS). Theoretically, it should be possible to use MAS to lower the cost and shorten the time to identify lines that carry the most desirable combination of genomic regions associated with resistance, quality, and yield. However, before MAS can be widely used in SCN breeding programs, resistance loci must be identified and cost-effective methods for marker analysis must be developed.

Based on previous results in our lab, the chromosomal locations of many SCN resistance loci are now known, including the most important on linkage group 'G' (Concibido *et al,* 1996a submitted). This gene is shared by at least five of the major sources of SCN resistance and can explain up to 50% of total variation in SCN resistance. In this paper, we present results of experiments to test the effectiveness of MAS using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers on linkage group 'G'. We also present preliminary results about a quick and simple marker analysis system based on the polymerase chain reaction.

Materials and Methods

Plant materials. One hundred and forty-seven F_5 lines from 16 distinct crosses in 1994 and 149 F_5 lines from 12 distinct crosses in 1995 were analyzed in this study. These advanced lines were chosen from the ongoing SCN breeding program at the University of Minnesota. The resistant parents used in this study include several widely-used sources of SCN resistance, 'Peking', PI 88788, PI 209332, PI 437654, or lines derived from them. The susceptible parents used in this study were Minnesota varieties with good agronomic traits but no known SCN resistance. Progeny from an F_6 recombinant inbred lines (RIL) 'Evans' x PI 209332 were also included in the analysis.

Leaf material for each line was collected in bulk either in the field or in the greenhouse for DNA extraction. DNA extraction, restriction digests, electrophoresis, Southern blots, hybridization, and autoradiography were performed according to methods described Concibido *et al*, (1994).

SCN Assay: For each line in the study, five F_5 or F_6 seeds were planted, inoculated and an SCN index calculated as previously described (Concibido *et al,* 1994). All greenhouse assays were inoculated with an SCN isolate that behaved as race 3. To ensure proper race identification, soybean differential lines ('Peking', 'Pickett', PI 88788, PI 90763, PI 209332) and the susceptible check Evans were planted and inoculated along with test. The greenhouse assay in 1994 was performed in water baths maintained at 28° C with a 16-hour daylength for 28 days. In 1995, assays were done on the greenhouse bench where temperatures fluctuated between 25° and 30° C.

Statistical and data analysis: Linkage mapping data were analyzed by Statview-II and Mapmaker-QTL software, as described in Concibido *et al,* (1994). Data were also analyzed by Statview-II software for contingency table analysis.

Results and Discussion

Major SCN Resistance Loci. The chromosomal location of a major partial resistance gene on linkage group 'G' has previously been identified in populations segregating for SCN resistance (Concibido *et al,* 1996a, 1996b). Discovery of this locus comes from analysis of four segregating soybean F2 populations ('Evans' x PI 209332, 'Evans' x PI 88788, 'Evans' x PI 90763, 'Evans' x 'Peking') and an F_6 RIL population derived from 'Evans' x PI 209332. Depending on the source of resistance, the genomic interval C6/Bng122 on linkage group 'G' explains between 26 and 50% of SCN disease response for race 3. This same genomic region was also found in PI 437654 (Webb *et al,* 1995). Now that this major partial resistance locus on linkage group 'G' has been identified, efforts are underway to further characterize and isolate markers in this genomic region. In addition to the putative resistance locus near C6/Bng122 on 'G', we have also identified a total of ten additional genomic regions associated with SCN resistance among the various resistant genotypes (Concibido *et al,* 1996b).

Effectiveness of MAS. To test the effectiveness of MAS in predicting SCN resistance, we selected a subset of lines from the onging SCN breeding program at the University of Minnesota. Since we already knew that the genomic region on linkage group 'G' was strongly correlated with SCN disease response, we genotyped the selected lines with RFLP markers C6 and Bng122 and contrasted these results with greenhouse SCN assays. Table 1 shows the results of two years' data comparing the phenotypic SCN disease response with genotype predictions based on markers C6 and Bng122. For our greenhouse assay, lines were considered highly or moderately resistant if their SCN index was less than 0.30, a cut-off breeders often use to select plants for advancing or crossing. (Schmidt *et al,* 1992). Using this criteria, MAS accurately predicted SCN disease response 75% of the time in 1994 and 79% of the time in 1995. In 1994, markers C6 and Bng122 predicted susceptibility 42 out of 48 times. Progeny from the F_6 RIL population showed comparable levels of accuracy (92%) when selection was based on the genotypes of RFLP markers C6 and Bng122. These results show that the resistance locus near C6/Bng122 is essential, but not sufficient by itself, for high levels of SCN resistance. However, our results are based on a relatively small number of lines, so this conclusion needs to be confirmed with further analysis.

Table 1: Comparison between predictions based on SCN phenotype versus marker genotype (C6/Bng122).

SCN Phenotype	Marker Genotype (C6/Bng122[a]) Advanced SCN Lines					
	1994			1995		
	R	S	Total	R	S	Total
Resistant (R)	18	6	24	18	4	22
Susceptible (S)	14	42	56	2	4	6
Total	32	48	80	20	8	28

[a]Only lines where the C6/Bng122 locus was informative

Although C6/Bng122 were good predictors of SCN disease response, some susceptible lines would have been retained, especially in the 1995 experiment. One possible explanation could have been the SCN assay. Greenhouse assays can be affected by many environmental factors. Temperature fluctuation could be a possible explanation for the low detection of susceptibility (50%) in 1995. Assays were run on the greenhouse bench during the summer, when temperature fluctuation could not be controlled.

For MAS to predict SCN response more precisely, it would be necessary to test markers near the other putative resistance loci. Nevertheless, the accuracy of MAS in predicting SCN disease response based on only C6/Bng122 in these populations indicates that MAS may soon be useful in "real world" breeding. Also significant are preliminary results from our F_6 RIL population. Here, the genomic region adjacent to the major partial resistance gene on linkage group 'G' harbors a quantitative trait locus for yield depression (Mudge *et al*, 1996). To use MAS, most effectively we will need to pinpoint the exact location of the yield depression locus and break its linkage with the SCN resistance allele.

RAPD Primers for MAS. In the future, MAS using PCR-based RAPD primers may make selection faster and less expensive. We found two primers, OPAG-10 and OPAM-10, that mapped to an area just above the resistance locus on 'G'. RAPD primer OPAM-10 was informative 90/134 times for the resistance locus in the 1994 advanced SCN lines (in other words, polymorphic between resistant and susceptible parents). We are continuing to screen RAPD primers to find markers closer to the resistance locus on 'G' that could possibly be used to screen soybean lines for the presence or absence of the major resistance gene (and select for recombinants with the nearby yield depression locus).

Since the efficiency of a breeding strategy is usually measured in terms of genetic gain (Fehr, 1987), as well as the related cost, we have performed a comparison between genotypic and phenotypic selection. To genotype 100 soybean lines with RFLP markers and current technology costs about $2.00 per data point. The entire process of DNA analysis, including DNA extraction, restriction digest, Southern blotting and autoradiography was estimated to take one person 8 days. To phenotype the same 100 lines using conventional SCN screening is a tedious and difficult process. The entire process involves two people and takes more than 30 days to accomplish, with the estimated cost per data point is $1.00. Although MAS using RFLP markers costs

slightly more than conventional screening, the accuracy and efficiency makes it an attractive alternative.

To successfully implement MAS into the SCN breeding programs, breeders need to be able to screen thousands of soybean lines quickly and at low cost. We are developing a protocol for a quick "micro-DNA" extraction that could be used with PCR-based primers. With this protocol it should be possible to screen thousands of lines in less than five days and quickly pick those that have the resistance locus for SCN. The "Generation DNA Purification Kit" (Gentra Systems, Inc. Minneapolis, MN) is a rapid, single-reagent system to extract small quantities of DNA from samples for PCR amplification originally developed for human DNA samples. DNA is collected on a collection card where it remains immobilized while proteins and other contaminants are selectively removed. The immobilized DNA on the collection card can be placed directly into a tube for RAPD reactions. Cost per data point for extraction, PCR, and data input is about $1.50. This method is much faster and easier than conventional screening methods, but at a slightly higher cost.

Acknowledgments

This research was supported by the Minnesota Crop Improvement Association (MCIA) and the Minnesota Soybean Research and Promotion Council. This article is published in the series of the Minnesota Agricultural Experiment Station. Results from this article may be cited without contacting the authors for permission, as long as a standard citation to the source of the work is included.

References

Concibido, V. C., R. L. Denny, S. R. Boutin, R. Hautea, J. H. Orf, N. D. Young. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Sci. 34:240-246.

Concibido, V. C., D. L. Lange, R. L. Denny, J. H. Orf and N. D. Young. 1996a. Genome mapping of soybean cyst nematode resistance genes in Peking', PI 90763, PI 88788 and PI 209332 using DNA markers. Crop Science (submitted).

Concibido, V. C., R. L. Denny, D. L. Lange, J. H. Orf and N. D. Young. 1996b. RFLP mapping and marker-assisted selection of soybean cyst nematode resistance in PI209332. Crop Science (submitted).

Fehr, W. R. 1987. Principles of cultivar development. Vol. 1 Theory and technique. McGraw-Hill. New York.

Mudge, J., V. C. Concibido, R. L. Denny, N. D. Young, J. H. Orf. 1996. Genetic mapping of a yield depression locus near a major gene for soybean cyst nematode resistance. Soybean Genetics Newsletter (1996).

Schmitt, D. P. and G. Shannon. 1992. Differentiating soybean to *Heterodera glycines* races. Crop Sci. 32:275-277.

Webb, D. E., B. M. Baltazar, A. P. Rao-Arelli, J. Schupp, K. Clayton, P. Keim, W. D. Beavis 1995 Genetic mapping of soybean cyst-nematode race-3 resistance loci in the soybean PI-437.654. Theor. Appl. Genet. 91:574-581.

Roxanne Denny [1] Joann Mudge [2]

Douglas Lange [1] James Orf [2]

Silvia Penuela [1] Nevin Young [1]

UNIVERSITY OF MISSOURI
Department of Agronomy
Columbia, Missouri 65211

Inheritance of resistance to *Heterodera glycines* races 1, 3, and 5 in Peking

Introduction

'Peking' is one of the most important sources in breeding for resistance to the soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) because it has genes for resistance to races 1, 3, and 5. Resistance to race 3 in Peking was reported to be conditioned by a combination of one dominant and two recessive genes (Rao Arelli, *et al.*, 1992). However, the inheritance of resistance to races 1 and 5 in Peking is still not clear. Information is not available to determine if loci giving resistance to race 1 are the same as those for race 3, or 5. Objectives were (1) to investigate the inheritance pattern of resistance to races 1, 3, and 5, and (2) to determine if loci conditioning resistance to race 1 also control resistance to race 3, 5 or both.

Materials and Methods

F_1 hybrids were obtained in 1992 from a cross between Peking, resistant to races 1, 3, and 5, and *cv.* 'Essex', susceptible to all known races. F_1s were selfed to produce an F_2 population and F_2 individuals were further used for generating $F_{2:3}$ families. F_2 and $F_{2:3}$ plants were planted in a greenhouse to screen for resistance to races 1, 3, and 5. Each $F_{2:3}$ family was divided into three subgroups, with five individuals from each of the $F_{2:3}$ families forming a subgroup. Two hundred-twenty F_2 individuals and 197 $F_{2:3}$ families were used to determine the reaction to race 1. We also screened 198 and 196 $F_{2:3}$ families for reaction to races 3 and 5, respectively.

Procedures for the greenhouse bioassay were based on that of Rao Arelli (1994). The index of parasitism (IP) was used to determine the reaction of each plant based on the system proposed by Golden *et al.* (1970).

$$IP = \frac{\text{No. of white females of a given individual}}{\text{Mean No. of cysts on susceptible Essex}} \times 100$$

All $F_{2:3}$ individuals were categorized as being resistant, segregating or susceptible. Chi-square (x^2) analysis was performed to test the goodness of fit to hypothesized genetic ratios.

Because all three $F_{2:3}$ subfamilies were derived from the same F_2 plant, it was possible to test whether individuals showing resistance to one race had the same reaction to another race. The SAS regression analysis was performed to determine relationships of the reaction of resistance between races 1 and 3, 1 and 5, and races 3 and 5 (SAS, 1990).

Table 1. Reactions of parents, F_2, $F_{2:3}$ families to SCN races 1, 3, and 5.

Generation and Races	Number of plants							Hypothesized Resistant Genes	Genetic Ratio	x^2	p
	Total	Observed			Expected						
		R*	Seg.	S	R	Seg.	S				
Race 1:											
Peking	35	35			35						
Essex	34			34			34				
F_2	220	10		210	30.94		189.06	RRr	9:55	16.49	<0.01
		10		210	10.3		209.7	Rrr	3:61	0.01	>0.90
		10		210	13.75		206.25	rr	1:15	220	<0.25
		10		210	41.25		178.75	Rr	3:13	38.2	<0.01
$F_{2:3}$	197	3	78	116	3.08	80.03	113.89	Rrr	1:26:37	0.33	>0.75
					12.31	98.5	86.19	Rr/rr	1:8:7	19.78	<0.01
Race 3:											
Peking	35	35			35						
Essex	35			35			35				
$F_{2:3}$	195	5	75	115	3.05	79.22	112.73	Rrr	1:26:37	0.47	>0.50
					12.19	97.50	85.31	Rr/rr	1:8:7	20.57	<0.01
Race 5:											
Peking	33	33			33						
Essex	34			34			34				
$F_{2:3}$	198	3	81	114	3.09	80.43	114.47	Rrr	1:26:37	0.05	>0.95
					12.38	99.00	86.63	Rr/rr	1:8:7	19.32	<0.01

* R = Resistant; Seg. = Segregating; S = Susceptible.

Results and Discussion

Reaction to race 1:

Two-hundred and twenty F_2 plants were used to screen for resistance to race 1. Ten plants were categorized as resistant and 210 were rated as susceptible which indicated that there were three genes, one dominant and two recessive, controlling resistance to SCN race 1 in Peking ($x^2 = 0.01$, $p > 0.90$). Data from 197 $F_{2:3}$ families confirmed the hypothesized three-gene pattern conditioning resistance to race 1. Three out of the 197 families were classified as resistant, 116 were susceptible, and 78 were segregating (Table 1). This segregation data fit the expected 1 (R) : 26 (Seg.) : 37 (S) ratio with a $x^2 = 0.33$, and $p > 0.75$.

Reaction to race 3:

There were a total of 195 $F_{2:3}$ families used to screen for resistance to race 3. Four out of 195 families were categorized as resistant, 115 were susceptible, and 76 were segregating. The segregation data fit the 1 (R) : 26 (Seg.) : 37 (S) ratio ($x^2 = 0.47$, p=0.50-0.75) (Table 1).

Reaction to race 5:

A total of 198 $F_{2:3}$ families were used to determine resistance to race 5. There were 3 resistant families, 114 susceptible, and 81 segregating, indicating that resistance to race 5 was conditioned by three genes (Table 1). These data were consistent with those from previous studies (Rao Arelli, 1995).

Because each $F_{2:3}$ subfamily came from the same F_2 plant, it allowed us to investigate whether the same set of genes controlled the reaction to all three races based on the SAS regression analysis. The coefficient of regression value for race 1 *vs.* race 5 was very low ($R^2 = 0.0262$, p=0.0276), indicating that Peking did not share the same set of genes with race 1 and that of race 5. Similarly, resistance loci to race 3 were inferred to be different from that of race 5 based on the coefficient of regression value ($R^2 = 0.0000$, $p = 0.9904$). However, regression coefficient between races 1 and 3 was relatively higher ($R^2 = 0.0805$, $p = 0.0001$), implying that Peking may have some similar gene(s) giving resistance to both races 1 and 3. Molecular marker analysis is currently underway to confirm these results.

References

Golden, A. M., J. M. Epps, R. D. Riggs, L. A. Fox, and R. L. Bernard. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Dis. Rep. 54: 544-546.

Rao Arelli, A. P., S. C. Anand, and J. A. Wrather. 1992. Soybean resistance to soybean cyst nematode race 3 is conditioned by an additional dominant gene. Crop Sci. 32: 862-864.

Rao Arelli, A. P. 1994. Inheritance of resistance to *Heterodera glycines* race 3 in soybean accessions. Plant Dis. 78: 898-900.

Rao Arelli, A. P. 1995. Allelic loci in Peking soybean controlling resistance to *Heterodera glycines* races 3 & 5. Soybean Gen. Newsl. 22: 170-172.

SAS Institute. 1990. SAS/STAT. User's Guide. Version 6.0, third edition. SAS Institute Inc. Cary, NC.

B. Qiu

D. A. Sleper

A. P. Rao Arelli

UNIVERSITY OF MISSOURI
Plant Science Unit
117 Curtis Hall
Columbia, MO 65211 USA

Soybean Germplasm Resistant to Races 1 and 2 of *Heterodera glycines*

Introduction

In the United States, *H. glycines* is the primary cyst nematode and is a most serious pest of soybean [*Glycine max* (L.) Merr.]. Several accessions were evaluated from soybean germplasm collections for resistance to this pest (Anand et. al. 1985; Ross and Brim, 1957; Rao Arelli et. al. 1992; Young, 1990, 1995; Nelson et. al. 1994). As of today, 118 resistant accessions are identified and these are primarily resistant to races 3, 5, and 14. Some of the data on their reaction to races 5 and 14 are inconclusive. Additionally, we have extremely very little available information on their reaction to races 1 and 2. The objective of this research was to initially bioassay 86 resistant accessions for reaction to *H. glycines* races 1 and 2 and also to reexamine them for other races.

Materials and Methods

Collection and preparation of near-homogeneous race isolates of *H. glycines* include the following:

Race 1 isolate: A field population of *H. glycines* was obtained from Washington County, NC. The females and cysts used for inoculum were first reproduced on the roots of susceptible cultivars Essex and Hutcheson for several generations to obtain a near-homogeneous population. This population was categorized as race 1 isolate based on the classification system of Riggs and Schmitt (1987) and maintained on the roots of Hutcheson.

Race 2 isolate: The methods described for race 1 isolate were employed to develop a near-homogeneous population of *H. glycines* from a field population collected from Beaufort County, NC. The population was reproduced and maintained on the roots of cv.Pickett and was classified as race 2 isolate.

Race 3 isolate: A field population of *H. glycines* collected from the roots of Essex was obtained from Ames Plantation, near Grand Junction, TN (courtesy of L. D. Young, USDA-ARS). The population was reproduced and maintained on the roots of Essex plants and was classified as race 3 isolate.

Race 5 isolate: A race 5 population was collected from a field grown to cv.Bedford on the Rhoades Farm of the University of Missouri near Clarkton, MO. The population was reproduced and maintained on the soybean PI line 88788 to obtain a near homogeneous population of *H. glycines* and that was classified as race 5 isolate.

Race 14 isolate: A race 14 field population was obtained from Orangeburg County, SC and was collected from the roots of

cv.Forrest. This population was reproduced on PI line 90763 to obtain a near-homogeneous population of race 14 isolate and maintained on cv.Hutcheson.

Development of Plant Material and Inoculation: Accessions were bioassayed from PI54591 through PI5676608 which included 86 out of 118 available resistant plant introductions. These were of all maturity groups and were collected from China, Japan, Russia and South Korea. A set of 86 soybean accessions plus Peking, PI90763, PI88788 and Pickett-71, the four host differentials and Essex and Hutcheson, the susceptible controls were included in each bioassay. These accessions were provided by R. L. Nelson, Curator of the USDA-ARS soybean germplasm collection. Bioassays were performed for each of the five *H. glycines* races according to the procedures described by Rao Arelli (1994). Each test included 20 replications.

Approximately 30 days after inoculation, plant roots were individually washed with a strong jet of water, females and cysts were counted under a steromicroscope and an Index of Parasitism (IP) or Female Index was calculated for each accession (Golden et.al. 1970).

$$\text{IP or Female Index} = \frac{\text{Average \# of cysts and females per accession}}{\text{Average \# of cysts and females per Essex or Hutcheson}} \times 100$$

Ratings of resistant (IP=0-9%), moderately resistant (IP=10-30%) moderately susceptible (IP=31-60%) and susceptible (IP=>60%) were used to classify the accessions (Schmitt and Shannon, 1992).

Results

All accessions bioassayed for *H. glycines* races 1 and 2 were PI54591 through PI567660B which included 86 lines (Table 1). Accessions PI54591 through PI17852B (Sel.) which included 53 lines were also bioassayed for races 3, 5, and 14 (Table 1). Thirty-three accessions, PI567285 through PI567660B were bioassayed for race 5 reaction (Table 1.). These 33 lines were recently reported resistant either to race 3 or 14 and/or both (Nelson, et.al., 1994).

These bioassays have identified 48 accessions with various levels of resistance to *H. glycines* race 1. These included 13 Resistant, 13 Moderately resistant, and 22 Moderately Susceptible soybean accessions. For race 2 reaction 44 soybean accessions were found resistant and these included; 10 resistant, 9 moderately resistant and 25 moderately susceptible. Among the recently identified 33 resistant accessions, we found 10 lines resistant to *H. glycines* race 5. These included 4 resistant, 2 moderately resistant and 4 moderately susceptible. (Table 1.)

The results also included 17 accessions with resistance to races 1, 2, 3, 5 and 14. (Table 1.) We are developing DNA molecular markers for the world soybean germplasm with resistance to *H. glycines* in collaboration with Southern Illinois University,Carbondale; and Asgrow Seed Company, Ames, Iowa.

A. P. Rao Arelli

J. A. Wilcox

References

Anand, S. C., J. A. Wrather, and C. R. Shumway. 1985. Soybean genotypes with resistance to races of soybean cyst nematode. Crop Sci. 25:1073-1075.

Rao Arelli, A. P., J. A. Wrather and S. C. Anand. 1992. Genetic diversity among isolates of *Heterodera glycines* and sources of resistance in soybeans. Plant Dis. 76:894-898.

Rao Arelli, A. P. 1994. Inheritance of resistance to *Heterodera glycines* Race 3 in soybean accessions. Plant Dis. 78:898-900.

Ross, J. P., and C. A. Brim. 1957. Resistance of soybeans to the soybean cyst nematode as identified by a double-row method. Plant Dis. Rep. 41:923-924.

Riggs, R. D., and D. P. Schmitt. 1988. Complete characterization of the race scheme for *Heterodera glycines.* J. Nematol. 20:392-395.

Schmitt, D. P., and G. Shannon. 1992. Differentiating soybean responses to *Heterodera glycines* races. Crop Sci. 32:275-277.

Young, L. D. 1990. Soybean germplasm evaluated for resistance to races 3, 5, and 14 of soybean cyst nematode. Crop Sci. 30:735-736.

Young, L. D. 1995. Soybean germplasm evaluated for resistance to races 3, 5, and 14 of soybean cyst nematode. Crop Sci. 35:895-896.

Golden, A. M., J. M. Epps, R. D. Riggs, L.A. Duclos, J. A. Fox and R. L. Bernard. 1970. Terminology and identity of infra-specific forms of the soybean cyst nematode *(Heterodera glycines).* Plant Dis. Rep. 54:544-546.

Nelson, R. L., R. G. Palmer, C. D. Nickell, G. R. Noel, R. C. Shoemaker and Y. W. Chen. 1994. Genetic diversity in soybean germplasm from central China. Agron. Abstr. p.83.

Table 1. Reaction and mean female indices (IPs) for 86 soybean accessions bioassayed for Race 1, 2, 3, 5 and 14 of *Heterodera glycines*.

S.No	Strain-designation	Race 1	Race 2	Race 3	Race 5	Race 14
1	PI54591	S* (67.0)**	S (74.0)	MS (41.0)	MR (30.0)	MS (40.0)
2	PI79609	S (74.0)	S (90.0)	MR (29.0)	MS (40.0)	MS (40.0)
3	PI79693	MR (29.0)	MS (35.0)	MR (14.0)	MR (30.0)	MR (12.0)
4	PI84751	R (3.0)	MR (28.0)	R (0.0)	R (1.0)	MR (14.0)
5	PI87631-1	MS (38.0)	S (67.0)	R (9.0)	MR (17.0)	MR (11.0)
6	PI88788	MS (39.0)	S (77.0)	R (5.0)	MS (46.0)	R (2.0)
7	PI89008	MS (35.0)	S (66.0)	MR (18.0)	MR (18.0)	MR (18.0)
8	PI89014	MS (31.0)	S (75.0)	MS (34.0)	MS (35.0)	MS (37.0)
9	PI89772	R (1.0)	R (3.0)	R (0.0)	R (1.0)	MR (14.0)
10	PI90763	R (8.0)	R (2.0)	R (0.0)	R (0.0)	MS (57.0)
11	PI91138	MS (45.0)	S (110.0)	MS (43.0)	MS (42.0)	MS (44.0)
12	PI92720	S (74.0)	S (84.0)	MR (25.0)	MS (56.0)	MS (34.0)
13	PI200495	MR (28.0)	MS (47.0)	MR (17.0)	R (4.0)	MR (12.0)
14	PI209332	MS (47.0)	S (70.0)	R (4.0)	R (7.0)	R (5.0)
15	PI303652	R (2.0)	MS (48.0)	R (1.0)	R (2.0)	MR (29.0)
16	PI339868B	R (2.0)	MR (20.0)	R (1.0)	R (2.0)	MR (13.0)
17	PI398680	MS (56.0)	S (79.0)	MR (26.0)	S (67.0)	MS (42.0)
18	PI398682	S (71.0)	MS (55.0)	MR (25.0)	MS (52.0)	MS (39.0)
19	PI399061	MS (38.0)	MS (42.0)	MR (33.0)	R (8.0)	S (64.0)
20	PI404166	R (1.0)	R (6.0)	R (1.0)	R (0.0)	MR (10.0)
21	PI404198A	R (1.0)	R (8.0)	R (1.0)	R (1.0)	MS (55.0)
22	PI404198B	R (2.0)	MS (44.0)	R (2.0)	R (1.0)	MR (24.0)
23	PI407729	MR (11.0)	MS (47.0)	MR (15.0)	R (6.0)	R (5.0)
24	PI407944	MS (60.0)	S (83.0)	MR (12.0)	S (63.0)	MS (57.0)
25	PI408192-2	MS (45.0)	S (84.0)	S (93.0)	MS (48.0)	MS (43.0)
26	PI416762	MR (17.0)	MS (47.0)	R (6.0)	R (6.0)	R (8.0)
27	PI417091	MR (20.0)	MS (55.0)	R (7.0)	R (4.0)	R (6.0)
28	PI417094	MS (33.0)	MS (30.0)	MR (13.0)	MR (11.0)	MR (23.0)
29	PI424137B	S (61.0)	S (88.0)	MS (35.0)	R (9.0)	MR (28.0)
30	PI 424595*	MS (58.0)	MS (45.0)	MS (50.0)	R (8.0)	MS (55.0)
31	PI437090	S (63.0)	S (107.0)	S (95.0)	MS (40.0)	MS (57.0)
32	PI437379	S (85.0)	S (102.0)	S (137.0)	MS (48.0)	S (70.0)
33	PI437488	MS (55.0)	S (85.0)	MS (34.0)	MS (44.0)	MR (28.0)
34	PI437654	R (0.0)	R (1.0)	R (0.0)	R (0.0)	R (0.0)
35	PI437655	R (1.0)	S (69.0)	R (1.0)	R (3.0)	MR (12.0)
36	PI437679	MR (22.0)	MR (14.0)	R (4.0)	R (0.0)	R (2.0)
37	PI437690	R (1.0)	R (7.0)	R (0.0)	R (2.0)	MR (25.0)
38	PI437725	R (1.0)	MR (19.0)	R (0.0)	R (2.0)	MS (46.0)

S.No	Strain-designation	Race 1	Race 2	Race 3	Race 5	Race 14
39	PI437770	MS (31.0)	S (61.0)	R (4.0)	MS (46.0)	MR (14.0)
40	PI437908	S (74.0)	S (81.0)	S (101.0)	MS (55.0)	S (85.0)
41	PI438183	MS (50.0)	S (93.0)	MR (21.0)	S (64.0)	MS (55.0)
42	PI438342	MS (38.0)	MR (22.0)	MS (42.0)	R (2.0)	MR (29.0)
43	PI438489B	R (0.0)	R (5.0)	R (1.0)	R (1.0)	R (8.0)
44	PI438496B	MR (30.0)	S (96.0)	R (2.0)	MS (35.0)	MS (41.0)
45	PI438497	R (5.0)	MR (17.0)	(NA) (NA)	R (2.0)	MR. (29.0)
46	PI438498	R (1.0)	MR (17.0)	R (4.0)	R (2.0)	MS (32.0)
47	PI438503A	MS (44.0)	MS (57.0)	R (3.0)	MR (22.0)	R (9.0)
48	Cloud PI16790 (Sel.)	MS (49.0)	S (61.0)	R (5.0)	MS (33.0)	MR (10.0)
49	Columbia	MR (17.0)	MS (35.0)	R (9.0)	MR (10.0)	R (8.0)
50	Ilsoy PI6387 (Sel.)	MR (29.0)	MS (49.0)	MR (14.0)	MR (18.0)	MR (14.0)
51	Patoka PI70218-2-19-3	S (132.0)	S (96.0)	S (106.0)	S (80.0)	MS (49.0)
52	Sooty	MS (37.0)	S (82.0)	MR (28.0)	MR (26.0)	MR (23.0)
53	PI17852B (Sel.)	R (2.0)	MS (58.0)	R (2.0)	R (1.0)	MS (51.0)
54	PI567285	S (100.0)	S (83.0)	R (NA)	S (82.0)	S (NA)
55	PI567286	S (116.0)	(NA) (NA)	R (NA)	S (81.0)	R (NA)
56	PI567303A	S (114.0)	(NA) (NA)	S (NA)	S (73.0)	R (NA)
57	PI567325B	S (128.0)	S (80.0)	R (NA)	S (99.0)	S (NA)
58	PI567328	S (115.0)	S (81.0)	S (NA)	S (112.0)	R (NA)
59	PI567336A	MR (16.0)	R (7.0)	R (NA)	R (2.0)	S (NA)
60	PI567336B	MS (32.0)	R (9.0)	R (NA)	R (2.0)	S (NA)
61	PI567432	MR (20.0)	R (5.0)	R (NA)	R (4.0)	S (NA)
62	PI567363B	S (81.0)	S (82.0)	R (NA)	S (66.0)	S (NA)
63	PI567364	S (115.0)	MS (42.0)	R (NA)	MS (45.0)	R (NA)
64	PI567365	S (94.0)	S (97.0)	R (NA)	MS (53.0)	S (NA)
65	PI567373A	S (93.0)	MS (56.0)	S (NA)	MR (17.0)	R (NA)
66	PI567373B	MS (60.0)	MS (35.0)	S (NA)	MS (43.0)	R (NA)
67	PI567400	S (77.0)	S (90.0)	S (NA)	S (98.0)	R (NA)
68	PI567415A	MR (29.0)	MR (24.0)	S (NA)	MR (17.0)	R (NA)
69	PI567418A	S (92.0)	MS (40.0)	S (NA)	S (89.0)	R (NA)
70	PI567421	S (95.0)	MS (36.0)	S (NA)	S (97.0)	R (NA)
71	PI567445	MR (15.0)	MR (27.0)	S (NA)	(NA) (NA)	R (NA)
72	PI567491A	R (0.0)	R (6.0)	R (NA)	R (0.0)	S (NA)
73	PI567492	S (103.0)	S (64.0)	S (NA)	S (94.0)	R (NA)
74	PI567507B	S (66.0)	S (66.0)	S (NA)	MS (36.0)	R (NA)
75	PI567510A	S (108.0)	S (71.0)	S (NA)	S (106.0)	R (NA)
76	PI567512B	S (94.0)	S (86.0)	S (NA)	S (160.0)	R (NA)
77	PI567516C	R (9.0)	R (8.0)	R (NA)	(NA) (NA)	S (NA)

S.No	Strain-designation	Race 1	Race 2	Race 3	Race 5	Race 14
78	PI567535A	S (100.0)	MS (45.0)	S (NA)	S (66.0)	R (NA)
79	PI567562A	S (116.0)	MS (50.0)	R (NA)	S (106.0)	S (NA)
80	PI567568A	MS (43.0)	S (61.0)	R (NA)	S (67.0)	R (NA)
81	PI567577	S (90.0)	S (67.0)	R (NA)	S (64.0)	S (NA)
82	PI567581	S (62.0)	MS (42.0)	S (NA)	S (61.0)	R (NA)
83	PI567583C	S (87.0)	MS (55.0)	S (NA)	S (113.0)	R (NA)
84	PI567583D	S (92.0)	S (68.0)	R (NA)	S (130.0)	S (NA)
85	PI567636	S (93.0)	MS (49.0)	R (NA)	MS (35.0)	S (NA)
86	PI567660B	S (64.0)	MS (34.0)	R (NA)	S (87.0)	S (NA)
87	cv.Peking	R (7.0)	S(63.0)	R (6.0)	R (8.0)	MS (60.0)
88	PI88788	MS (56.0)	S (68.0)	R (3.0)	S (61.0)	R (9.0)
89	PI90763	R (1.0)	R (1.0)	R (1.0)	R (2.0)	MS (60.0)
90	cv.Pickett-1	R (2.0)	S (68.0)	R (7.0)	S (71.0)	S (69.0)
91	cv.Essex	S (100)	S (100)	S (100)	S (100)	S (100)
92	cv.Hutcheson	S (100)	S (100)	S (100)	S (100)	S (100)

* Reaction Rating; ** Index of Parasitism or Female Index; NA=Not Available

NORTH CAROLINA STATE UNIVERSITY [1]
Department of Crop Science
USDA-ARS
Raleigh, North Carolina 27695-7631

PIONEER HI-BRED INTERNATIONAL INC. [2]
PO Box 1004
Johnston, IA 50131

Can Heterosis Be Predicted by Genetic Similarity Measures in Soybean?

INTRODUCTION:

Choosing parents is one of the most challenging aspects of plant breeding. There can be several criteria in the selection of parents when breeding for high yield. Two of the most common criteria are high yield per se, and genetic distance between parents. Yield is relatively easy to measure with field evaluations, but the genetic relationship is not because of complicated pedigrees. Two measures of genetic distance have been proposed. One is the coefficient of parentage (CP) or coancestry, based on pedigree analysis and defined as the probability that a random gene from one individual is identical by descent to a random gene of another. The other is the RFLP-based genetic similarity estimate (RFLP-GS), which is defined as the proportion of RFLP bands identical between two genotypes, compared to the total number of bands. It is important to determine whether these two measures of genetic distance are only theoretical concepts or have real meaning for the breeder in the selection of parents. One way to validate the utility of genetic similarity measures is in the prediction of the genetic variance among the progeny (inbred lines) in a biparental cross situation. There is some evidence that crossing parents with low CP (less genetic relationship), increases the genetic variance among inbred lines in soybean (Manjarrez-Sandoval, 1996).

A second method of validating the usefulness of genetic similarity measures is the prediction of heterosis. Although there is no current use for heterosis in commercial soybean production, male-sterility and cytoplasmic male-sterility systems have been developed for production of hybrids. Thus, the information generated here may have a potential use in future soybean production. The objectives of this paper are to (i) estimate the mid-parent heterosis for yield and other agronomic characteristics in a range of soybean cultivars adapted to the Southern US, and (ii) empirically test the predictive value of coefficient of parentage and RFLP-based genetic similarity estimates for midparent heterosis.

MATERIAL AND METHODS:

Twenty four F2 populations were developed by crossing three tester parents ('Young', 'Centennial' and 'Tracy') to eight assorted parents. The combination of each tester with eight parents was designated a set. For each set, the eight parents were cultivars representing a wide range of CP and RFLP-GS with respect to the tester; and, thus, were a good sampling of the diversity available among southern US soybean cultivars (Table 1). Each combination of tester, parent and F2 bulk was designated a cross. The CP among parents were calculated according to Carter et al. (1993). The protocols for obtaining RFLP autoradiograms have been previously described by Keim et al., (1988) and Keim et al., (1989). We used 33 RFLP probes representing 47 polymorphic markers loci well distributed throughout the genome. The RFLP-GS estimates were calculated according to Nei and Li (1979).

The field study to evaluate F2 midparent heterosis was planted on June 1 and 6, 1994 at Clayton and Plymouth, NC, respectively. The experimental design for each set in each location was a split-plot with eight replications. The whole plots were the eight crosses. Subplots were assigned randomly to the three genotypes in each cross- the two parents and the F2 bulk. To assure the identity of the F2 populations, each row in an F2 plot was planted using seed from a different F1 plant and segregation patterns were examined. We detected only eight F2 center rows as selfs, in which case one of the plot border rows was harvested as a substitute for yield evaluation. The midparent heterosis was determined in the F2 rather than the F1 generation due to the impracticality of producing a large amount of F1 seed for testing. Seed yield, days to maturity, and 100-seed weight were recorded. Maturity was recorded only for parents; all other variables were measured on all plots. No heterogeneity of error variances was detected among cross, set and locations. Initially each set was analyzed separately. Subsequently, an overall analysis was performed over locations and sets.

RESULTS AND DISCUSSION:

The agreement between CP and RFLP-GS was good; the correlation between these measures was 0.80, 0.92 and 0.95 in sets one, two and three, respectively, and all were highly significant ($p<0.01$). Thus CP and RFLP-GS gave similar estimates of genetic distance between parents in this study.

Midparent heterosis for yield across the two locations was 8.5, 4.8, and 8.4% for sets one, two and three, respectively. Midparent heterosis was 3.5. 1.6, and 3.0% for 100-seed weight, and 4.1, 5.4, and 13.1% for plant height in sets one, two and three, respectively (heterosis values were significant at the $\alpha=0.05$ level, except 100-seed weight in sets two and three and plant height in set one). The range of mid-parent heterosis for yield, 100-seed weight and plant height that we found is similar to other reports in soybean (Gizlice et al., 1993; Dan Dyer, 1991, personal communication; Nelson and Bernard, 1984).

Table 1. Coefficient of parentage(CP), RFLP-based genetic similarity estimates(RFLP-GS), and midparent heterosis for yield(YIELD), 100-seed weight (SDWT) and plant height(HEIGHT) in percent over the two locations, and in absolute values for yield in each location.

Cross			OVER LOCATIONS			Clayton NC	Plymouth NC
			HETEROSIS				
Tester x Parents	CP	RFLP-GS(%)	YIELD†	SDWT†	HEIGHT†	YIELD‡	YIELD‡
			---------%---------			------- kg/ha ------	
			Set 1				
Young x Tracy	.103	70	9.8	7.6	7.1	228	295
Young x York	.156	70	6.3	3.9	3.2	120	217
Young x Dyer	.188	61	15.1	9.7	13.8	518	268
Young x Vance	.277	62	10.2	-0.2	6.6	138	373
Young x Toano	.307	73	4.7	0.9	-0.58	241	41
Young x TN 5-85	.364	77	12.0	4.4	3.2	285	371
Young x Sohoma	.424	81	3.6	2.6	2.7	140	34
Young x Essex	.555	88	6.4	-0.8	-2.7	435	-104
Mean			**8.5**	**3.5**	**4.1**	**263**	**187**
LSD (0.05)			**9.9**	**3.6**	**6.0**	**401**	**371**
			Set 2				
Centennial x Davis	.074	61	4.5	5.4	7.5	16	201
Centennial x Hutcheson	.154	62	6.9	5.0	11.9	203	186
Centennial x Toano	.222	63	10.5	1.9	10.2	458	121
Centennial x Tracy	.281	74	6.7	2.5	3.2	200	160
Centennial x Bedford	.374	78	5.0	-0.7	7.3	93	163
Centennial x Forrest	.429	82	2.3	0.32	6.1	161	-19
Centennial x Leflore	.688	84	1.1	0.67	0.24	55	-8
Centennial x Twiggs	.714	85	1.7	-2.7	-3.4	299	-188
Mean			**4.8**	**1.6**	**5.4**	**186**	**77**
LSD (0.05)			**7.9**	**4.3**	**5.8**	**299**	**342**
			Set 3				
Tracy x Davis	.027	57	8.1	4.7	9.6	127	303
Tracy x Hutcheson	.102	62	7.7	1.5	8.9	343	126
Tracy x Essex	.178	67	8.6	4.8	19.0	92	387
Tracy x Bedford	.237	74	7.7	4.5	8.2	218	199
Tracy x Forrest	.271	76	11.0	2.5	13.5	378	259
Tracy x Pickett-71	.317	78	11.4	5.0	8.3	469	159
Tracy x Dyer	.378	79	4.5	0.49	28.0	97	100
Tracy x Sharkey	.640	87	8.5	0.37	9.7	227	198
Mean			**8.4**	**3.0**	**13.2**	**244**	**216**
LSD (0.05)			**9.1**	**3.2**	**7.9**	**425**	**308**

† Midparent heterosis over the two locations, in percent.
‡ Midparent heterosis by location, in absolute values (kg/ha).

Neither CP nor RFLP-GS completely predicted heterosis for yield when averaged over the two locations, in part due to a rather large GXE interaction in the heterosis estimation. Individual location and set analyses revealed that heterosis could be predicted from either CP or RFLP-GS at Plymouth but not at Clayton for sets one and two. In set three, heterosis could not be predicted either by CP or RFLP-GS at either location. Thus, the relationship between CP or RFLP-GS with respect to heterosis for yield was location and population dependent.

The lack of relationship between CP or RFLP-GS with heterosis in Clayton could not be attributed to the experimental conditions in this location. Relatively high yields were observed in both locations. We looked for high residuals on plot basis but no outliers were detected. A relatively high precision was obtained in the estimation of heterosis in our study due to the eight replications employed. Contradictory heterotic responses across environments have been reported before (Nelson and Bernard, 1984; Cox and Murphy, 1990). One possible explanation for our observed GXE in seed yield heterosis is based in the proposition of Compton (1977) that heterosis in self-pollinated species may be explained mainly by additive x additive epistasis. A few major genes interacting in one location but not in the other could cause the differential expression of heterosis between locations. However, the lack of prediction of heterotic effect in set three was not due to a larger than average GXE interaction.

The relation of yield heterosis to maturity difference between parents was not useful in explaining any heterosis trends because it was not consistent among sets. In set one we found an inverse relationship between heterosis for yield and the difference in maturity between parents, while in set two we observed a direct relationship between the variables. In set three there was no relationship.

GXE was not important in the estimation of F2 heterosis for 100-seed weight and plant height, and both were predicted reasonably well by CP and RFLP-GS in the first two sets. We examined the relationship between heterosis for yield and the other variables but found no consistent relations. Heterosis for yield was correlated with plant-height heterosis in sets one and two at Plymouth, but not at Clayton. In set three, no correlation between heterosis for the different characters was found.

CONCLUSION:

CP and RFLP-GS were only partially effective in predicting heterosis for yield, 100-seed weight and plant height. Thus, one should not restrict selection of parents for heterosis studies to genetic distances measures only. The GXE in the estimation of heterosis for yield may be larger than in the normal evaluation of breeding lines. This means that if in the future one would want to commercialize soybean hybrids, one may expect a more extensive than normal yield evaluation over environments that for inbreds. Because the large magnitude of GXE in the estimation of heterosis for yield in our study, suggest that additional environments (locations and years) may improve the estimates of relationship between heterosis and measures of genetic distance.

REFERENCES

Carter, T. E. Jr, Z. Gizlice, and Burton, J. W. 1993. Coefficient-of-parentage and genetic similarity estimates for 258 North American public cultivars released between 1945 and 1988. U.S. Dep.of Agric.Tech.Bull. Num. 1814, U. S. Gov. Print. Office, Washington, DC.

Compton, W. A. 1977. Heterosis and additive x additive epistasis. Soybean Genetics Newsletter 4:60-62.

Cox, T. S. and Murphy J. P. 1990. The effect of parental divergence on F2 heterosis in winter wheat crosses. Theor. Appl. Genet. 79:241-250.

Gizlice, Z., T. E. Carter Jr., and J. W. Burton. 1993. Genetic diversity in North American soybean: II. Prediction of heterosis in F2 populations of southern founding stock using genetic similarity measures. Crop Sci. 33:620-626.

Keim, P., R. Groose, and R. S. Shoemaker. 1988. A rapid protocol for isolating soybean DNA. Soybean Genet. Newsletter 15:150-152.

Keim, P., R. C. Shoemaker, and R. G. Palmer. 1989. Restriction fragment length polymorphism diversity in soybean. Theor. Appl. Genet. 77:786-792.

Manjarrez-Sandoval, P. 1996. Genetic variance among inbred lines and F2 heterosis as predicted by coefficient of parentage and RFLP-based genetic similarity estimates in soybean. Ph.D.Dissertation. NCSU, Crop Science Department. Raleigh, NC.

Nei M. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad.Sci. 76:5269-5273.

Nelson, R. L. and Bernard, R. L. 1984. Production and performance of hybrid soybeans. Crop Sci. 24:549-553.

P. Manjarrez-Sandoval[1]

T.E. Carter Jr. [1]

D.M. Webb[2]

J.W. Burton[1]

NORTH CAROLINA STATE UNIVERSITY [1]
Department of Crop Science
USDA-ARS
Raleigh, North Carolina 27695-7631

PIONEER HI-BRED INTERNATIONAL INC. [2]
PO Box 1004
Johnston, IA 50131

Coefficient of Parentage and RFLP Markers: Are They Useful in Predicting Genetic Variance in Soybean Populations?

INTRODUCTION

Coefficient of parentage (CP) and RFLP genetic-similarity estimates (RFLP-GS) have been proposed as measures of genetic distance to study patterns of diversity within crop species. The coefficient of parentage, also called coancestry, is defined as the probability that a random gene from one individual is identical by descent to a random gene of the other individual. RFLP-GS is defined as the proportion of RFLP bands identical, compared to the total number of bands between two cultivars. In soybean, RFLP-based markers have been efficient in detecting genotypic differences among soybean cultivars, corresponding to genetic differences in the pedigree (Shoemaker, *et al.*, 1992; Keim *et al.*, 1989). However practical application of these measures in plant breeding remains uncertain. Their ultimate utility depends on their predictive value for parameters of interest, such as genetic variance among inbred lines. The present research was designed to probe the relationship of the CP and RFLP-GS between parents and the genetic variance among their inbred progeny.

MATERIALS AND METHODS

We chose to study five biparental populations selected to represent a wide range CP and RFLP-GS between parents (Table 1). Inbred lines were developed by single seed descent method (SSD) over the past 15 years, as a part of the USDA-ARS soybean breeding program for high yield at North Carolina State University, Raleigh, NC. The CP among parents were calculated according to Carter *et al.* (1993). The protocols for obtaining RFLP autoradiograms have been previously described by Keim *et al.*, (1988) and Keim *et al.*, (1989). We used 33 RFLP probes representing 47 polymorphic markers loci well distributed throughout the genome. The RFLP-GS estimates were calculated according to Nei and Li (1979). During the summer 1993, seeds were retrieved from cold storage and random inbred lines were increased for each population. In 1994 enough seed were available to evaluate 30 inbred lines from each population.

The five populations were planted during the spring of 1994 at Clayton and Plymouth, NC on June 1 and 6, respectively. The experimental design was a split-split plot, with sub-subplots nested within each subplot. The main plots were sets and the subplots were the five populations. Six random lines were nested within each population-set combination. Locations, replications, sets and lines were considered random effects, while populations were considered fixed. Sets were used to lessen effects of soil heterogeneity on population comparisons. Three replications were grown at each location. The genetic

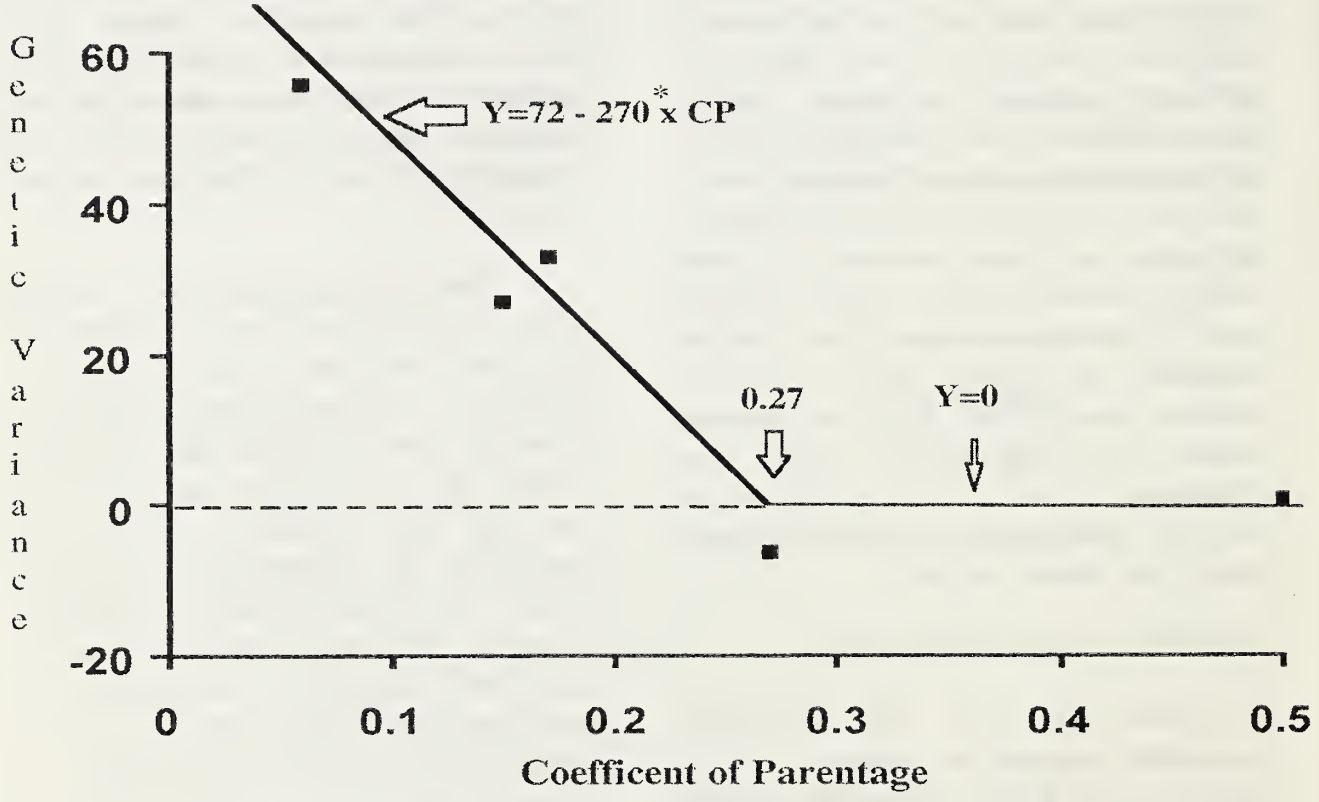
variance among inbred lines by population was computed from variance component estimates obtained from the analysis of variance. An overall analysis was performed over locations and sets. For this analysis, some lines were discarded because they had either, very low plant stand, large residuals or large differences in maturity. The final number of lines included in each population are presented in Table 1. After these deletions, neither heterogeneity of error variance nor maturity effects were a significant factor in the study.

RESULTS AND DISCUSSION

CP and RFLP-GS as predictors of genetic variance for yield.

The general experimental conditions were excellent, with mean yields in Clayton and Plymouth, NC of 3440 and 2969 kg/ha, respectively. Results indicated that CP was a more efficient predictor of genetic variance (GV) for yield among inbred lines, than was RFLP-GS. A clear and significant inverse relationship between CP and GV was detected (Figure 1), when data were fit to a linear-plateau model. According to the model, the GV was calculated as zero when the CP was larger than 0.27. The importance of this fact is that 0.28 is the average CP for the southern soybean cultivars released between 1979-1988 (Gizlice *et al.*, 1993), and frequently parents with higher CP than 0.28 are crossed in a practical breeding program. A consequence is that many breeding populations may have little value for producing significantly higher yielding progeny.

Figure 1. Relationship between coefficient of parentage and genetic variance for yield (x1000) among inbred lines in five populatiuons evaluated in two North Carolina locations during the 1994 summer.



* significant (alpha=0.05)

RFLP-GS was a less effective predictor of GV than CP. Lower GV was found both at the lowest and the highest values of RFLP-GS (Table 1). This disagrees with the CP-based results and with our initial hypothesis. However, these results were highly influenced by population four. When population four was deleted and results reanalyzed, the inverse correlation between RFLP-GS and GV was high ($r_{RFLP\text{-}GS.GV}$ =-0.93, p=0.06).

Table 1. Characteristics of five soybean populations tested at two locations in NC in 1994.

Populations No.	Pedigree	CP[©]	RFLP-GS[©©]	No.[©©©] lines	Genera-tion [♣]	GV[¶]	SE GV[¶¶]	Matu-rity [¶¶¶]
1	Davis x N73-1102[■]	.06	58	20	$F_{6:8}$	55838	31168	27
2	Young x N73-1102	.15	65	28	$F_{8:10}$	26852	18697	21
3	Forrest x Bay	.17	59	27	$F_{8:10}$	32853	20641	17
4	N77-179[♦] x Forrest	.27	46	23	$F_{6:8}$	-6100	10879	14
5	Essex x Vance	.50	74	22	$F_{6:8}$	760	12825	18

[©] Coefficient of Parentage
[©©]RFLP-based Genetic-Similarity estimates
[©©©]Final number of inbred lines included in each population
[♣] Filial generation at the evaluation time
[■] **N73-1102**=Tracy x Ransom
[♦]**N77-179**=N70-1549 x N72-3213
[¶] Genetic variance among inbred lines for yield
[¶¶] Standard error of genetic variance
[¶¶¶] October 1st=1.

The correlation between CP and RFLP-GS for the five populations in our study was 0.45, which was not statistically significant (p=0.43). The low correlation was due primarily to population 4 which had a high CP value (0.27), and a low RFLP-GS estimate (46%). Otherwise, CP and RFLP-GS generally agreed. In other experiments, Manjarrez-Sandoval (1996) found a correlation of 0.66 between CP and RFLP-GS in 24 pairwise comparisons of soybean cultivars. In maize, correlations from 0.71 to 0.9 between CP and RFLP-GS have been reported (Messer *et al.*, 1993, Smith *et al.*, 1990). Thus, we believe that the correlation between these genetic similarity estimates would have been higher in the present study if we had examined a greater number of parents. We believe that the 33 RFLP probes used in our study were efficient in estimating genetic similarity between genotypes. Previously, a correlation between genetic-similarity estimates based on these 33 RFLP probes and 78 additional (111 in total) RFLP probes, in 30 soybean cultivars was 0.92 (David Webb, unpublished data).

Expected gains

One may use our results to calculate expected gain from selection for the five populations. If one assumes that the breeder will select the single highest yielding line from 100 lines evaluated in the field (for a given population), and that the evaluation takes place in three locations, with two replications per location, then the expected gain for populations 1, 2, and 3 was larger

than for populations 4 and 5 (Table 2). Populations 1, 2 and 3 were the three with the lowest CP between parents. Adding the expected gain to the mean yield for each population, we obtain the expected yield of the best line by population. The expected yield of the best line is larger for populations 1 and 2 than for the rest (Table 2), again indicating that populations with low CP between parents gave an advantage in selection.

Table 2. Heritability and expected genetic gain for yield in five soybean populations, assuming are evaluated in three locations with two replications in each location

Populations		h^{2}	Δ_G	Mean yield	Predicted yield
No.	Parents			(kg/ha) ‡	(kg/ha) ‡‡
1	Davis X N73-1102	0.61	480	3079	3559
2	Young X N73-1102	0.43	285	3281	3566
3	Forrest X Bay	0.48	331	3153	3484
4	N77-179 X Forrest	0.00	0	3469	3469
5	Essex X Vance	0.02	11	3272	3283

Heritability (on entry mean basis)

$h^2 = \sigma^2_G / [(\sigma^{2'}_e / lr) + (\sigma^2_{GXE}/l) + \sigma^2_G]$

$\sigma^{2'}_e = \sigma^2_e + \sigma^2_{RxP(L\,S)} + \sigma^2_{RXS(L)}$

$\sigma^2_{GXE} = \sigma^2_{RXG(L)}$

Expected genetic gain (kg/ha/cycle)

$\Delta G = i.h.\sigma_G$

i=2.508, based on selecting 1 in 100 lines.

l=locations; g=lines; r=replications; p=populations; s=sets; h=(heritability)$^{1\backslash 2}$.

‡ Actual mean of lines in each population.

‡‡ Predicted mean yield of best line = (Mean yield) + (ΔG for one cycle of selection).

The assessment of genetic gain for the five populations may seem somewhat theoretical and removed from applied breeding. One way to validate our results is to trace the actual fate of breeding lines in a breeding program. The five populations in this study were developed and tested in an applied breeding program in North Carolina. If CP and GV were important in breeding, then one would expect that populations with a high GV and low CP would produce a higher frequency of lines in various stages of yield testing. For the USDA-ARS breeding program at NCSU, the testing stages are single plot yield trials (Plant Rows), replicated tests at two locations in NC (Local Preliminary Test), regional testing at eight locations (Regional Preliminary Test), and finally regional tests at 30 locations (Uniform Test). Our expectations agreed with the actual fate of breeding lines. Populations 1 and 2, those with higher GV and lower CP, contributed a larger percentage of lines to advanced testing stages than did populations 4 and 5 (Table 3). In fact, the cultivar Brim was derived from Population 2.

Table 3 . The fate of breeding lines developed for practical improvement in the USDA-ARS soybean breeding program in Raleigh, NC over a period of 13 years.

Populations		Year in	No.Lines	No. Lines	No. Lines	No. Lines
No.	Parents	PR[©]	in PR[©]	in LP[©©]	in RP[#]	in UT[♣]
1	Davis x N73-1102	1987	74	11	3	1
2	Young x N73-1102	1982-83	201	21	2	1
3	Forrest x Bay	1982	168	3	0	0
4	N77-179 x Forrest	1987	67	5	0	0
5	Essex x Vance	1989	255	40	0	0

[©] Plant Rows.
[©©] Local preliminary test
[#] Regional preliminary test
[♣] Uniform test

CONCLUSIONS

This is the first demonstration of the inverse relationship between the genetic variance for yield among inbred and the coefficient of parentage of the parents in soybean. Our results validate the practical application of the coefficient of parentage in the selection of parents for high-yield-population development. In our study, the genetic variance among inbred lines was calculated as zero when the CP of the parents was larger than 0.27, roughly equivalent to half-sib relatives in soybean. The predictive value of CP with respect to GV, indicates that in the case of soybean, the assumptions involved in the calculation of CP (no genetic relationship among ancestors, no genetic drift due to selection, and 50% of contribution of each parent in a biparental cross) seem to be reasonable (St. Martin, 1982; Lorenzen *et al.*, 1995; Gizlice *et al.*, 1993).

REFERENCES

Carter, T. E. Jr, Z. Gizlice, and J. W. Burton. 1993. Coefficient-of-parentage and genetic similarity estimates for 258 North American public cultivars released between 1945 and 1988. U.S. Dep.of Agric.Tech.Bull. No.1814, U. S. Gov. Print. Office, Washington, DC.

Gizlice, Z., T. E. Carter Jr., and J. W. Burton. 1993. Genetic diversity in North American soybean: I. Multivariate analysis of founding stock and relation to coefficient of parentage. Crop Sci. 33:614-629.

Keim, P., R. Groose and R. S. Shoemaker. 1988. A rapid protocol for isolating soybean DNA. Soybean Genet. Newsletter 15:150-152.

Keim, P., R. C. Shoemaker, and R. G. Palmer. 1989. Restriction fragment length polymorphism diversity in soybean. Theor. Appl. Genet. 77:786-792.

Lorenzen, L. L., S. Boutin, N. Young, J. E. Specht and R. C. Shoemaker. 1995. Soybean pedigree analysis using map-based molecular markers: I. Tracking RFLP markers in cultivars. Crop Sci. 35:1326-1336.

Manjarrez-Sandoval, P. 1996. Genetic variance among inbred lines and F2 heterosis as predicted by coefficient of parentage and RFLP-based genetic similarity estimates in soybean. Ph.D. Dissertation. NCSU, Crop Science Department. Raleigh, NC.

Messmer, M. M., A. E. Melchinger, R. G. Herrmann, and J. Boppermaier. 1993. Relationships among early European maize inbreds: II. Comparison of pedigree and RFLP data. Crop Sci. 33:944-950.

Nei M. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad.Sci. 76:5269-5273.

Shoemaker, R. C., R. D. Guffy, L. L. Lorenzen, and J. E. Specht. 1992. Molecular genetic mapping of soybean: Map utilization. Crop Sci. 32:1091-1098.

Smith, O. S., J. S. C. Smith, S. L. Bowen, R. A. Tenborg, and S. J. Wall. 1990. Similarities among a group of elite maize inbreds as measured by pedigree, F_1 grain yield, heterosis, and RFLPs. Theor. Appl. Genet. 80:833-840.

St. Martin, S. K. 1982. Effective population size for the soybean improvement program in maturity groups 00 to IV. Crop Sci. 22:151-152.

P. Manjarrez-Sandoval[1]
T. E. Carter Jr. [1]
D. M. Webb[2]
J. W. Burton[1]

NORTH CAROLINA STATE UNIVERSITY
Department of Crop Science
Raleigh, NC 27695-7631

Soybean Seedling Grafting Technique

Grafting experiments have been used to gain further understanding of the control of root and shoot systems in soybean plants. For example, Carver et al. (1987) demonstrated that root stock significantly influenced seed fatty acid composition of grafted scions. Caldwell and Hanson (1968) reported that seed protein and oil accumulation were governed by the above ground portion of the plant. Cho and Harper (1991) used reciprocal grafting experiments to study the response of nodulation mutants in soybean. They found that the nonnodulating mutant NN5 was strictly root controlled, whereas the hypernodulating mutant NOD1-3 was shoot controlled.

We have been interested in studying the root system of the Japanese plant introduction PI 416937. This plant introduction has an unusual fibrous root morphology that contributes to its drought tolerance, aluminum tolerance, and enhanced nitrogen fixation capacity (Hudak and Patterson, 1995; Sloane et al., 1990, Goldman et al., 1989, Pantalone, 1995). In order to demonstrate the beneficial effects of the fibrous root morphology, we initiated large-scale grafting studies to provide sufficient numbers of grafted seedlings to conduct row-planted experiments in the field. The purpose of this paper is to report the procedure used to successfully generate soybean seedling grafts.

Materials and Methods

Soybean seeds were planted in Jiffy® peat pellets. Grafts were initiated five days after planting. At that time the seedlings had emerged and reached a height of approximately four cm from soil surface to apical meristem. The hypocotyl was transversely severed with a razor approximately two cm below the cotyledon (Figure 1). The lower portion remained in the soil to become the root stock. The upper portion became the scion. A vertical incision was made with a razor to a depth of approximately 0.75 cm into the top center of the root stock. The scion from another seedling was razor-trimmed to a v-wedge which was inserted into the root stock incision. The graft union was wrapped with parafilm. A 12 cm length bamboo skewer was set into the soil to provide support. Additional parafilm was used to secure the graft to the bamboo skewer (Figure 2). Grafts were placed out of direct sunlight, under greenhouse benches. The seedling grafts were hand-watered through a fine-mist nozzle every hour for the first 12 hours then four times daily for four days. Grafts were then brought to the top of the greenhouse benches for three days. At that time, plants were transplanted to the field.

Results and discussion

The use of peat pellets provided ease of maneuverability when grafting seedlings and transplanting them to field plots. The vertical incision in the root stock provided a snug fit of the scion wedge graft. Parafilm secured the union firmly and reduced desiccation of grafted tissue. Misting the seedling grafts further reduced desiccation and allowed rapid healing of the graft wound. The bamboo skewers provided initial support in the field and helped to prevent grafts from splitting at the union during adverse weather conditions. This seedling grafting methodology resulted in a 90% success rate and is useful to generate large numbers of grafts for field research.

References

Caldwell, B. E. and W. D. Hanson. 1968. Relative importance of stem and root genotype in determining differences in percent protein and oil of soybean seed. Crop Sci. 8:629-630.

Carver, B. F., J. W. Burton, and R. F. Wilson. 1987. Graft-transmissible influence on fatty acid composition of soybean seed. Crop Sci. 27:53-56.

Cho, M. J. and Harper, J. E. 1991. Root isoflavonoid response to grafting between wild-type and nodulation-mutant soybean plants. Plant Physiol. 96:1277-1282.

Goldman, I. L., T. E. Carter, Jr. and R. P. Patterson. 1989. Differential response to drought stress and subsoil aluminum in soybean. Crop Sci. 29:330-334.

Hudak, C. M. and R. P. Patterson. 1995. Vegetative growth analysis of a drought-resistant soybean plant introduction. Crop Sci. 35:464-471.

Pantalone, V. R. 1995. Utilization of a fibrous rooted plant introduction for soybean improvement. Ph.D. Diss. North Carolina State Univ.

Sloane, R. J., R. P. Patterson, and T. E. Carter, Jr. 1990. Field drought tolerance of a soybean plant introduction. Crop Sci. 30:118-123.

V. R. Pantalone

J. W. Burton

T. E. Carter, Jr.

Figure 1. Grafting of soybean seedlings in peat pellets.

Figure 2. Sealing soybean seedling graft union with parafilm onto bamboo skewer.

USDA-ARS, SOIL DRAINAGE
RESEARCH UNIT AND
THE OHIO STATE UNIVERSITY
590 Woody Hayes Drive
Columbus, OH 43210

Optimization of Silver-Staining AFLP Technique for Soybean

Introduction

During the past few years, the use of the polymerase chain reaction-based technique has significantly increased the application of DNA markers to genotyping, genome mapping and phylogenetics.

The Amplified Fragment Length Polymorphism (AFLP) technique was invented in 1993 (Zabeau, 1993). Its advantage over other DNA marker techniques includes the detection of a large number of polymorphism from a single PCR reaction. This technique requires the use of radioactive isotopes for primer labeling. In addition, the use of streptavidin beads for DNA template selection is tedious and only produces a small amount of DNA. Since the patent, several companies have obtained the right to develop application kits for AFLP. The kits are now available, but most of the information about the methodology is proprietary and is not available to individual researchers. Using the original information from the patent, we have optimized the technique for silver-staining AFLP. This technique also incorporated the pre-selective PCR step (Lin & Kuo, 1995), thus eliminating the use of streptavidin beads for DNA template selection. We report here our experience in using this technique on five soybean genotypes.

Materials and Methods

Plant Materials. The plants of 2 adapted high-yielding soybean lines (A83-271027 and Conrad) and 4 PI strains (PI 297.502, PI 339.864A, PI 347.541, and PI 360.844) were grown in the growth chamber at 27°C temperature with a 16-h photoperiod at 800 μ mol photon m^{-2} s^{-1} for six weeks. The PI were from China, South Korea, Romania, and Japan, respectively.

DNA Extraction. DNA was extracted from one plant of each line by the CTAB method (Saghai-Maroof et al., 1984).

Synthesis of Oligonucleotide Adapters and Primers. The *EcoR*I and *Mse*I adapters and primers specified by Zabeau (1993) were synthesized by Ransom Hill Bioscience, Inc. (Ramona, CA).

DNA Restriction and Ligation. Restriction and ligation were done concurrently by adding 2.5 μg genomic DNA to 60 μl buffer (10 mM Tris-HCl, pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT) containing 20 units *EcoR*I, 16 units *Mse*I, 17

pmol *EcoR*I adapter, 170 pmol *Mse*I adapter, 1 unit T_4 DNA ligase and 0.1 µM ATP. The mixture was incubated at 37°C for 4 h.

Labeling of Primers. When radioactive isotope was used, the *Mse*I primer was end-labeled with either ^{32}P or ^{33}P-γATP as described by Zabeau (1993).

DNA Amplification. We used two consecutive PCRs to selectively amplify the *EcoR*I-*Mse*I DNA fragments. The pre-selective amplification (first PCR) contained in 25 µl reaction mixture (10 mM Tris-HCl, pH 8.3, 50 mM KCl) 6 µl of 10-fold diluted restriction and ligation products, 40 ng of *Mse*I and *EcoR*I primers each, 2 mM $MgCl_2$, 2 mM dNTPs, and 2 units Taq polymerase. The PCR program was 94°C for 60 sec, followed by 20 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 60 sec.

The selective amplification (second PCR) was similar to the pre-selective amplification except 40 cycles of PCR at 62°C annealing temperature were used and the primers had three selective bases.

Gel Electrophoresis. The PCR products were mixed with an equal volume (25 µl) of loading buffer containing 98% formamide, 10 mM EDTA, 0.01% (w/v) bromo phenol blue, 0.01% (w/v) xylene cyanol. The mixture was incubated in boiling water for 3 minutes and cooled on ice before 6 µl was loaded to each lane of a 5% denaturing polyacrylamide sequencing gel (8 M urea). The gels were run at 80 watts constant power for 90 min. Gels for autoradiography were washed with d. water containing 10 % (v/v) acetic acid and 10 % (v/v) methanol for 30 min, and dried overnight at room temperature.

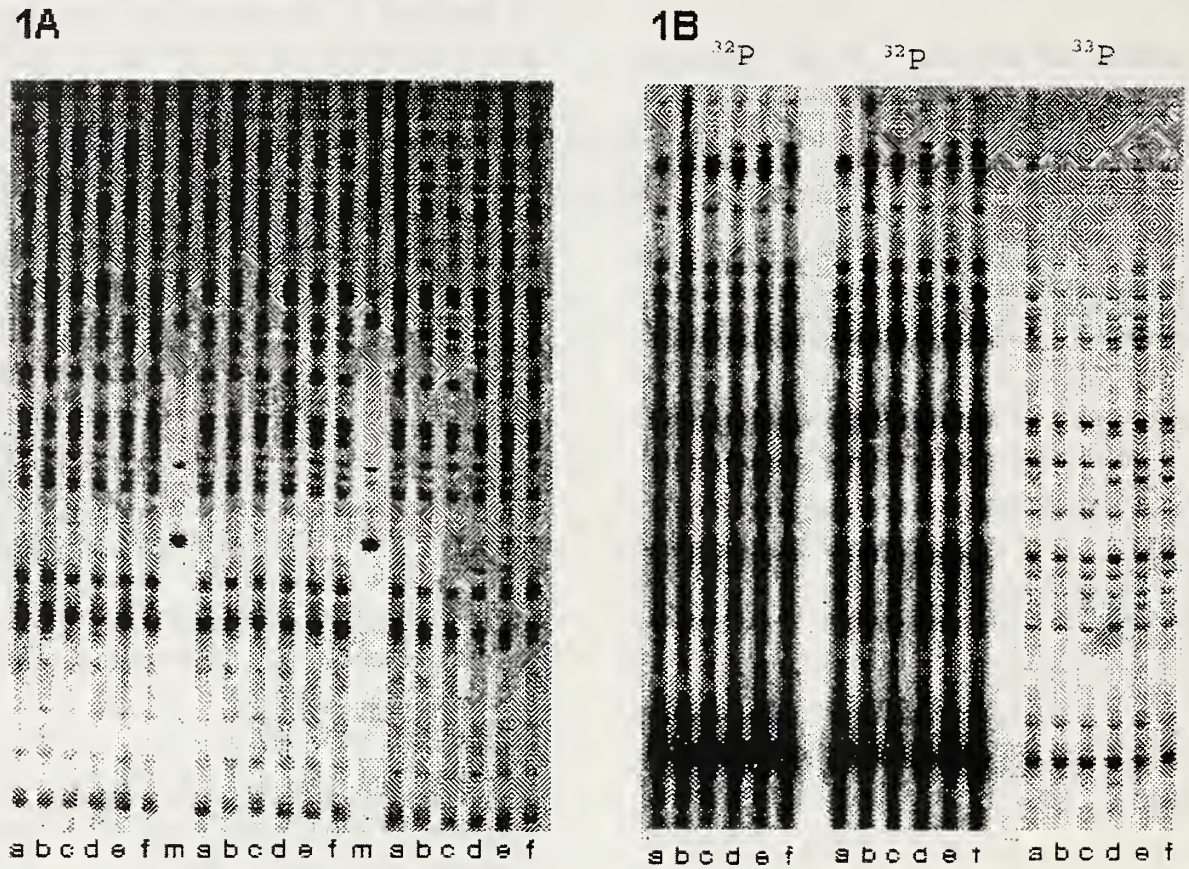
Silver Staining. Silver staining was done as described by Bassam et al., (1991). The gels were first fixed in 7.5% acetic acid for 30 min. It was then washed three times with a large quantity of d. water for 5, 3 and 2 min, respectively. The gel was transferred to a silver impregnation solution (1.5 g/L $AgNO_3$, 0.056 % formaldehyde) for 30 min, followed by a 5-sec rinse with d. water. All of the above steps were done with slow agitation on an automatic shaker. The image development step was done with manual agitation for 1 to 2 min in a developer solution (30 g/L Na_2CO_3, 0.056% formaldehyde, 400 µg/L sodium thiosulfate) followed by a 1 min fix in 7.5 % acetic acid. The gel was then rinsed briefly in d.water and dried at room temperature.

Results and Discussion

Reproducibility of the AFLP results. The results of silver-staining AFLP of DNA from the six soybean lines in three independent experiments are shown in Figure 1a. Identical results were obtained with primers that were either labeled with ^{32}P or ^{33}P (Figure 1b). While ^{33}P is more expensive, it provides higher resolution.

In our experience, the AFLP technique is much more affected by the reproducibility of the thermoprofile during and between experiments than the RAPD technique. Our earlier efforts in performing AFLP using an MJR (Boston, MA) thermocycler produced variable results even with two reactions from the same master mix in the same PCR experiment. By using thermocouples in individual PCR reactions,

Figure 1. Silver-staining (1A) and ^{32}P and ^{33}P AFLP (1B) gels of six soybean lines a, A83-271027; b, Conrad; c, PI 297.502; d, PI 339.864A; e, PI 347.541; f, PI 360.844; and m, 1 kb molecular weight marker.



we traced the problems to the reproducibility of the thermoprofile within and between experiments. In some cases, a discrepancy of as much as 2°C was detected. We have been able to overcome the thermoprofile reproducibility problem by using block temperature control (in place of probe temperature control), no heated lid, oil-cover PCR reactions and by increasing the number of steps in the thermocycle program. These extra steps were necessary to keep the targeted temperature and duration consistent in all reaction vials within and between experiments. Using the above modified procedures, we have repeatedly obtained consistent results . Our experience underscored the necessity of confirming the thermoprofile reproducibility within and across experiments.

Silver Staining AFLP. One advantage of silver-staining AFLP is the elimination of hazardous radioactive isotopes. However, this method requires more labor and skill in processing the gels than the use of radioactive isotope labeling. The resolution of silver-stained AFLP gels is comparable with that of ^{33}P gels (Figure 1a & 1b). Both, however, have considerably higher resolution than ^{32}P gels. Photography of silver stained

gels by exposing the gels to Kodak APC paper for 3 min of fluorescent ceiling light while convenient is expensive. We were able to directly scan silver-stained gels using a flat bed scanner. Copies of the gels can also be made using standard photocopy machines. In terms of cost, silver staining and ^{32}P are much less expensive than ^{33}P. Each PCR reaction using ^{32}P or silver staining costs about $1.00 as compared with $ 3.00 for ^{33}P.

AFLP Markers. Using one pair of primers, we identified on the average 8 polymorphic bands between two adapted and four PI soybean strains. The maximum number of polymorphic bands detected was 14 (VanToai et al., 1996). This technique is therefore, much more efficient than the RFLP (Helentjaris, 1987) and RAPD (Williams et al., 1990) techniques.

Summary

The AFLP technique is a very efficient DNA marker technique. Using a single primer pair, we were able to identify 14 polymorphic bands among two adapted and four PI strains. Since Taq polymerase is expensive, the high number of polymorphism detected makes this technique more cost efficient than the RAPD technique. The technique is sensitive to the reproducibility of the thermoprofile within and across experiments. Silver-staining AFLP, while eliminating the use of radioactive isotopes, requires more skilled labor than the ^{32}P or ^{33}P-AFLP technique, and certainly more skilled labor than the RAPD technique.

Tara T. VanToai
Jiqing Peng
Steve St. Martin

REFERENCES

Bassam, B. J., G. Caetano-Anolles, and P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80-83.

Helentjaris, T. 1987. A genetic linkage map for maize based on RFLPs. Trends Genet. 3:217-222.

Lin, J. J. and J. Kuo. 1995. AFLP. A novel PCR-based assay for plant and bacterial DNA fingerprinting. Focus 17:66-70.

Saghai-Maroof, M. A., K. M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 81:8014-8018.

VanToai, T. T., J. Peng and S. Martin. 1996. Using AFLP markers to determine the contribution of parental genomes during recurrent selection. Soy. Gen. News. 23:

Williams, J. G., A. R. Kuberlik, K. J. Livak., J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6231-6235.

Zabeau, M. 1993. Selective restriction fragment amplification: A general method for DNA fingerprinting. European Patent Office. Publication number 0 534 858 A1.

USDA-ARS, SOIL DRAINAGE RESEARCH UNIT AND
OHIO STATE UNIVERSITY
590 Woody Hayes Drive
Columbus, OH 43210

Screening for Flooding Tolerance of Soybean

Introduction

We are interested in using molecular plant breeding to improve the flooding tolerance of soybean. This technique requires the mapping of QTL and identification of DNA markers closely linked to the flood-tolerant trait. An efficient and reproducible screening technique is essential for QTL mapping. The majority of flooding research in the literature was conducted by complete submersion of seedlings 3- to 7-d-old in deoxygenated buffer for 1 to 4 days. At the end of the stress, the seedlings were allowed to grow and their viability determined. This type of screening technique can be done rather quickly and does not require a lot of space. The extrapolation of such screening results to field flooding tolerance has not been substantiated. In this study, we report the results of the field, greenhouse and laboratory screening tests of 21 soybean lines of different flood-tolerant levels in our collection.

Materials and Methods

Plant materials. The 21 soybean lines in this study are listed in Table 1. All the seeds were from the 1994 season. Seed quality of all lots was high with germination being at least 90%.

Laboratory screening for tolerance to complete submergence. Seed disinfection and germination were by the method of VanToai et al., (1995). Healthy, unblemished 3-d-old seedlings were sorted for uniformity in size and used in the submergence treatment.

The seedlings were submerged in flooding buffer (10 mol m^{-3} Tris-HCl, pH 8.0, 100 g/L ampicillin) in air-tight glass jars (0.95 L) for 48 h. After the submergence treatment, seedlings were placed between two layers of germination paper soaked with 2 mol m^{-3} captan and allowed to grow in the dark at 27±1°C for three days. The appearance of fleshy white roots was used as the criterion for seedling viability. The experiment was repeated three times, each with 50 seedlings per replicate.

Field screening for tolerance to root flooding. The seeds were planted by hand on 15 May 1995 in 5-ft rows at 20 seeds per row. Herbicides were applied as needed. No fertilizer was needed as determined by soil tests. The experimental design was a randomized complete block for the flooded and control treatments. Each soybean line was replicated four times. At three weeks after planting, the flooded treatment was begun by irrigating continuously with drip

irrigation to create surface ponding during the entire growing season. The non-flooded control treatment was irrigated as needed.

Greenhouse screening for tolerance to root flooding. Soybean seeds were planted in 4"x4"x4" plastic pots. At three weeks after planting, the plants were flooded with tap water in large stainless steel tubs (23"x56"x12 ") such that the water level was between 1/2" to 1 " above the soil surface. Flooding continued until harvest (12 weeks).

Determination of flooding tolerance. Leaf color was determined at the end of the growing season under both field and greenhouse conditions with a SPAD meter (Minolta, Ramsey, NJ). Seeds were harvested and threshed by hand at the end of the season.

Results and Discussion

Tolerance to complete submergence. The survival of seedlings after two days of complete submergence is reported in Table 1. Soybean seedlings do not grow under complete submergence. Tolerant plants survive submergence better than susceptible plants and are capable of resuming vigorous root and shoot growth after 2 days of complete submergence. Susceptible plants are either dead or only show minimum secondary root growth under the same stress. Tolerance to complete submergence is a short term reaction, which, in soybean, lasts less than three days. We consider this tolerance an innate ability that occurs mainly at the biochemical level (Sachs et al., 1980) and allows the seedlings to survive the stress.

Tolerance to root flooding. Differences in the tolerance to root flooding among the lines were detected under both field and greenhouse conditions (Table 1). Tolerant soybean varieties show vigorous shoot and root growth, green leaves, normal plant height and a large number of functional nodules during prolonged flooding treatment from seedling to maturity stages (12 weeks). Susceptible soybean varieties are chlorotic, stunted , and have many dead roots without nodules. In soybean, the supply of nitrogen essential for crop growth and seed production is derived primarily from nitrogen fixation. The energy for nitrogen fixation is generated by high rates of nodule respiration (Layzell and Hunt, 1990; Purcell and Sinclair, 1995) that requires oxygen. Tolerant soybeans, therefore, must have the ability to either transport large amounts of oxygen to nodules or to fix nitrogen efficiently under low oxygen environments. We have observed that tolerant soybean varieties develop extensive lenticels, adventitious roots and aerenchyma for oxygen transport. Such ability is less well developed in susceptible soybean varieties. The mechanism of tolerance to root flooding in soybean, therefore, differs from the mechanism of tolerance to complete submergence mentioned above. Tolerant plants develop an adaptation ability to transport oxygen to the roots for continuous growth during extended periods of root flooding.

The leaf color, as determined by SPAD reading, of both field and greenhouse plants appeared to correlate well with the seed yield reduction under flooding stress.

The correlation between tolerance to complete submergence (survival) and tolerance to root flooding was 0.7 for

greenhouse plants (leaf color) and 0.5 for field plants (seed yield). Some lines (Xu U.S.) were very tolerant to complete submergence but did not produce much yield under soil flooding. The problem was related to the late maturing of this line. Most plants grew much better under greenhouse flooding than under field flooding. Since greenhouse plants were grown in autoclaved soil, the interaction between flooding tolerance and disease tolerance is more prominent under field conditions. To thrive under field flooding, soybean is required to tolerate not only excessive soil water but also diseases that are prominent in flooded soils.

Summary

Soybean lines differ in their tolerance to both complete submergence and root flooding. Tolerance to complete submergence is an innate ability that occurs at the biochemical level and allows the seedlings to survive short durations of submergence. Tolerance to root flooding depends on the adaptation ability of the plants to develop mechanisms for transportation of oxygen to the roots. Tolerant plants not only survive, but also grow normally during extended periods of root flooding. The correlation coefficient of tolerance to submergence and tolerance to root flooding under greenhouse conditions was 0.7 and tolerance to field flooding was 0.5. Under field conditions, tolerance to diseases that are prevalent in flooded soil may play an important role in the growth and productivity of soybean.

References

Layzell D. B., Hunt S. (1990). Oxygen and the regulation of nitrogen fixation in legumes nodules. Physiol Plant 80:322-327.

Purcell L. C., Sinclair T. R. (1995). Nodule gas exchange and water potential response to rapid imposition of water deficit. Plant Cell Environ 18:179-187.

Sachs, M. M., Freeling M., and Okimoto R. (1980). The anaerobic proteins of maize. Cell 20:761-767.

VanToai T. T., Saglio P., Ricard B., and Pradet A. (1995). Developmental regulation of anoxic stress tolerance in maize. Plant, Cell and Environment 18:937-942.

Tara T. Vantoai

N. Nurjani

Table 1. Tolerance of 21 soybean lines to complete submergence and soil flooding

Names	FY/P (g)	CY/P (g)	FY/CY	FLCR	GHLCR	S S (%)
Williams	13.28	42.00	0.32	0.64	0.85	58
Nizhen	4.08	18.02	0.23	0.70	0.97	75
Pella	10.28	32.84	0.31	0.73	1.05	58
Conrad	6.81	28.85	0.24	0.71		76
Resnik	11.19	25.83	0.43	0.73	0.90	56
Sloan	10.48	29.34	0.36	0.74	0.99	65
Chapman	10.11	33.29	0.30	0.76	0.82	60
Edison	16.66	23.04	0.72	0.74	0.87	
Williams82	12.99	45.68	0.28	0.67	0.88	61
TaiLake Black	5.93	7.77	0.76	0.78	1.08	78
Sui Dao Huang	10.65	33.02	0.32	0.69	0.86	55
Na 54 Huai	18.07	18.61	0.97	0.72	1.02	80
Xu US	4.26	15.36	0.28	0.81	0.97	84
Daibangchin	0.67	12.57	0.05	0.77	0.80	14
Flyer	14.37	41.80	0.34	0.78	0.95	55
Thorne	8.54	39.68	0.22	0.68	0.89	57
Vertex	5.68	19.72	0.29	0.78		74
Sandusky	4.13	30.25	0.14	0.68		
General	13.50	41.46	0.33	0.67	0.82	
Ia2007	6.58	28.90	0.23	0.74	0.92	
Kenwood	10.89	26.24	0.41	0.76	0.90	

FY/P: average seed yield of flooded field plants (g)
CY/P: average seed yield of control field plants (g)
FY/CY: yield ratio of flooded by control field plant
FLCR: leaf color ratio of flooded by control field plants
GHLCR: leaf color ratio of flooded by control greenhouse plants
SS: submergence survival of seedlings (%)

USDA-ARS, SOIL DRAINAGE RESEARCH UNIT AND
THE OHIO STATE UNIVERSITY
590 Woody Hayes Drive
Columbus, OH 43210

Using AFLP Markers to Determine the Contribution of Parental Genomes during Recurrent Selection.

Introduction

Differential contribution of parental genomes during germplasm enhancement by recurrent selection is an important question in plant breeding. During the past few years, the use of the polymerase chain reaction-based technique has significantly increased the application of DNA markers to genotyping, genome mapping and phylogenetics. The Amplified Fragment Length Polymorphism (AFLP) technique was invented in 1993 (Zabeau, 1993). Its advantage over other DNA marker techniques includes the detection of a large number of polymorphisms from a single PCR reaction. We report here our experience in using this technique to determine the contribution of parental genomes during recurrent selection.

Materials and Methods

Plant Materials. The soybean population HS10 was developed in 1985 from 6 crosses between 2 adapted high-yielding soybean lines (A83-271027 and Conrad) and 4 PI strains (PI 297.502, PI 339.864A, PI 347.541, and PI 360.844). The PI were from China, South Korea, Romania, and Japan, respectively. Additional intermating was achieved by backcrosses and three-way crosses in 1986 in such a way that about 75% of the gene pool of the original population was contributed by the two adapted parents. The 14 best C0 lines were selected in 1989 based on yield, maturity, and lodging. Recombination of the 14 lines was carried out in 1990 and the 10 best C1 lines were selected for a second recombination cycle that was done in 1994.

Seeds of the 6 parents, the 14 C0 selections and 10 C1 selections were used in this study. For DNA extraction, the plants were grown in the growth chamber at 27 C temperature with a 16-h photoperiod at 800 μ mol photon m^{-2} s^{-1} for six weeks.

DNA Extraction. DNA was extracted from one plant of each parental line and five individual plants representing each of the 14 C0 selections and 10 C1 selections by the CTAB method (Saghai-Maroof et al., 1984).

Synthesis of Oligonucleotide Adapters and Primers. The *EcoR*I and *Mse*I adapters and primers specified by Zabeau (1993) were synthesized by Ransom Hill Bioscience, Inc. (Ramona, CA).

DNA Restriction and Ligation. Restriction and ligation were done concurrently by adding 2.5 μg genomic DNA to 60 μl buffer (10 mM Tris-HCl, pH 7.5, 10 mM MGgAc, 50 mM KAc, 5 mM DTT) containing 20 units *EcoR*I, 16 units *Mse*I, 17 pmol *EcoR*I adapter, 170 pmol *Mse*I adapter, 1 unit T_4

DNA ligase and 0.1 μM ATP. The mixture was incubated at 37 C for 4 h.

Labeling of Primers. When radioactive isotope was used, the *Mse*I primer was end-labeled with either ^{32}P or ^{33}P-γATP as described by Zabeau (1993).

DNA Amplification and Gel Electrophoresis DNA amplification and gel electrophoresis were done as described by VanToai and Peng (1996).

Statistical Analysis. We examined gel lanes of parents for bands that differed among the parents (polymorphic bands). If there was any doubt about the presence or identity of a band in the parents or progeny, the band was not used in this analysis.

If the five progeny of a given selection all showed a band, the selection was considered homozygous for that band. If all five progeny lacked the band, the selection was considered homozygous for the absence of the band. If some progeny had the band and others lacked it, the original selection was considered heterozygous.

The frequency of each polymorphic band among C0 and C1 selections was determined as y = (D + _H)/N, where D and H are, respectively, the number of selections homozygous and heterozygous for the band, and N is the total number of selections (N=14 for C0, N=10 for C1).

The contributions (p_1, p_2, p_3, p_4, p_5, and p_6) of each parent line to the C0 and C1 selections were estimated using the matrix equation $\mathbf{Y} = \mathbf{X}\beta + \epsilon$, where $\mathbf{Y}$ is the vector of frequencies of each band in the selections, β is the vector of p_j values to be estimated, and ϵ is a vector of error terms. The matrix $\mathbf{X}$ is an incidence matrix for parental bands such that $x_{ij} = 1$ if parent j has band i and $x_{ij} = 0$ otherwise. For each cycle, estimates and standard errors for β were obtained by ordinary least squares, with the constraint $\Sigma p_j = 1$.

Results and Discussion

Classification of selections based on a relatively small sample of progeny (5 plants) may lead to occasional misclassification. Plants from which DNA was extracted were four selfing generations removed from the original selected plant. Therefore, assuming complete dominance of bands, a heterozygous selection would produce 5 progeny having the band with a probability of $(17/32)^5 = 0.042$. For a heterozygous selection to produce 5 progeny lacking the band, the corresponding probability is $(15/32)^5 = 0.023$. Thus, about 93.5% of heterozygous selections should be classified correctly, and misclassifications should not have large effects on the estimation of parental contribution.

Using one pair of primers, we identified 14 polymorphic bands. In the absence of large random samples of plants from segregating populations, it was impossible to determine whether a pair of bands was inherited independently. Each pair of bands was checked to determine whether a relationship of complete dependence existed. A pair of bands was considered completely dependent if one band was always present whenever the other was lacking. No pairs of completely dependent bands were identified, so all bands were treated as if independent.

The estimate of parental contribution in the C0 and C1 population is presented in Table 1. After two cycles of recurrent selection for high yield, maturity and lodging it was surprising that the contribution of the two adapted parental lines in the C0 and C1 population remains essentially unchanged at 75 to 80 % .

Table 1. Contribution of parental genomes during recurrent selection.

	Population			
	C0		Cl	
Parental Lines	Contribution (%)	Std. Error (%)	Contribution (%)	Std. Error (%)
A83-271027	**21.18	6.76	**22.74	8.94
Conrad	**58.69	6.84	**51.91	9.07
PI 297.502	**11.54	4.13	**16.24	5.47
PI 339.864A	6.26	6.22	4.58	8.26
PI 347.541	-1.15	7.57	1.43	10.04
PI 360.844	3.85	5.66	3.10	7.51

** Highly significant as determined by the F-test.

Summary

The AFLP technique is a very efficient DNA marker technique. Using a single primer pair, we were able to identify 14 polymorphic bands in a soybean breeding population selected recurrently. Since Taq polymerase is expensive, the high number of polymorphism detected makes this technique more cost efficient than the RAPD technique. With one single primer pair we were able to show that the contribution of the two adapted parental genomes remains essentially unchanged after two cycles of recurrent selection and that up to 25 % of the gene pool of the C0 and C1 populations were still derived from the PI genomes.

Tara T. VanToai
Jiqing Peng
Steve St. Martin

References

Saghai-Maroof, M. A., K. M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 81:8014-8018.

VanToai, T. T. and J. Peng. 1996. Optimization of silver-staining AFLP technique for soybean. Soy. Gen. News. 23: ?-?.

Zabeau, M. 1993. Selective restriction fragment amplification: A general method for DNA fingerprinting. European Patent Office. Publication number 0 534 858 A1.

CLEMSON UNIVERSITY
Department of Agronomy
Department of Biological Sciences
Clemson, South Carolina

Clone pBLT65 is Associated with Soybean Cyst Nematode (SCN) Race 1 Resistance in J87-233

The germplasm soybean line J87-233 (maturity group V) is resistant to soybean cyst nematode (SCN) races 1, 2, 3 and 5 with moderate resistance to race 14, as well as root knot nematode (Young, 1993). It derives SCN resistance from three primitive soybean plant introductions: PI 88788, PI 90763, and Peking. Hutcheson is a high yielding maturity group V cultivar that is susceptible to SCN (Buss et al. 1988). The $F_{2:3}$ progeny population of the cross J87-233 x Hutcheson has been used to characterize the molecular profile of SCN resistance in J87-233. DNA molecular markers specific for SCN resistance have been identified in several primitive sources widely used in soybean breeding programs (Concibido et al. 1994; Mahalingam and Skorupska 1995; Webb et al. 1995). Two cDNA clones, pBLT24 and pBLT65, gave polymorphisms with TaqI and HaeIII, respectively, that have been shown to be very tightly linked to the i locus conferring self dark seed coat color (Weisemann et al. 1992). A quantitative trait locus for SCN race 3 resistance was localized in close proximity to the i locus (Mahalingam and Skorupska, 1995; Webb et al. 1995).

Probe pBLT24 is a clone encoding a soybean seed protein with a high degree of homology to known thiol protease sequences, and pBLT65 is a λgt11 library clone detected by hybridization with a carrot aspartokinase homoserine-dehydrogenase (Weisemann et al. 1992). Both clones were used in RFLP analysis of J87-233, Hutcheson, and progeny genotypes. Probe pBLT24 was found to be monomorphic for all five restriction enzymes used in this study, but pBLT65 gave polymorphic hybridization patterns between J87-233 and Hutcheson for three restriction enzymes, and was found to be significantly associated with SCN race 1 resistance.

Materials and Methods

Genomic DNA was isolated from leaf material of J87-233, Hutcheson, and 125 $F_{2:3}$ progeny genotypes, restriction digested with EcoRI, EcoRV, DraI, HindIII, and TaqI, Southern transferred and then hybridized using methods previously described by Skorupska et al. (1994). The SCN bioassays were performed at the University of Missouri, Columbia, MO using a waterbath method as previously described by Rao-Arelli et al. (1988). Linear regression analyses were conducted using SAS, version 6.10 (SAS Inst., 1991).

Results and Discussion

Southern hybridization of genomic DNA with probe pBLT65 showed polymorphisms between J87-233 and Hutcheson for enzymes EcoRI, EcoRV and TaqI. For digestions with EcoRI, three major bands were detected in J87-233, and four were detected in Hutcheson. For digestions with EcoRV, three major bands were detected for J87-233 while only two were detected for Hutcheson. For digestions with TaqI, five bands were detected for J87-233 and six were detected for Hutcheson. Approximate sizes of the detected bands are given in Table 1. The EcoRV polymorphism had the most discrete hybridization pattern. The observed segregation of the pBLT65/EcoRV polymorphism gave good fit to a 3:1 ratio in the progeny population, with the J87-233 phenotype (presence of the 1,700 bp band) being dominant. The polymorphism pBLT65/EcoRV was also linked (7.7 cM, LOD 3.0) with pK400/EcoRI polymorphism.

Significance of marker association with SCN race 1 resistance was established by linear regression of pBLT65 molecular phenotypic data with SCN bioassay data for the 125 $F_{2:3}$ progeny genotypes. Probe pBLT65 described 11.2% of the variation for race 1 resistance. The polymorphism described 3.6% of the variation for race 5. No significant association was found for pBLT65 and race 2 resistance.

Table 1. Approximate sizes of major bands detected by pBLT65 in southern hybridizations of genomic DNA (bp)

EcoRI		EcoRV		TaqI	
J87-233	Hutcheson	J87-233	Hutcheson	J87-233	Hutcheson
14000	14000	11500	11500	10500	10500
11500	13000	6000	6000	2600	3100
9800	11500	1700	1600	1900	2500
	9800	1600	1200	1800	1900
		1200		1600	1800
					1700

References

Buss, G. R., H. M. Camper, Jr., and C. W. Roane. 1988. Registration of 'Hutcheson' soybean. Crop Sci. 28:1024.

Concibido V. C., R. L. Denny, S. R. Boutin, R. Hautea, J. H. Orf, and N. D. Young. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Sci. 34:240-246.

Mahalingam, R., and H. T. Skorupska. 1995. DNA markers for resistance to *Heterodera glycines* I. race 3 in soybean cultivar Peking. Breeding Sci. 45:435-443.

Rao-Arelli, A. P. and S. C. Anand. 1988. Genetic relationships among soybean plant introductions for resistance to race 3 of soybean cyst nematode. Crop Sci. 28:650-652.

SAS Institute, Inc. 1991. User's guide: Statistics 6.10. SAS Inst., Cary, NC.

Skorupska, H. T., I. S. Choi, A. P. Rao-Arelli, W. C. Bridges. 1994. Resistance to soybean cyst nematode and molecular polymorphism in various sources of Peking soybean. Euphytica. 75:63-70.

Webb, D. M., B. M. Baltazar, A. P. Rao-Arelli, J. Schupp, K. Clayton, P. Keim, and W. D. Beavis. 1995. Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean PI 437.654. Theor Appl Genet. 91:574-581.

Weisemann, J. M., B. F. Matthews, and T. E. Devine. 1992. Molecular markers located proximal to the soybean cyst nematode resistance gene, Rhg4. Theor Appl Genet. 85:136-138.

Young, L. D. 1993. Registration of J87-233 soybean germplasm with resistance to soybean cyst nematode races 1, 2, 3, and 5. Crop Sci. 33:348.

J. A. Heer
E. R. Shipe
A. P. Rao-Arelli
H. T. Skorupska

CLEMSON UNIVERSITY
Departments of Agronomy and Horticulture
Poole Agricultural Center, Clemson, SC 29634

Growth Responses of Long-Juvenile Soybean Near-Isoline Pairs to Different Spectral Balances of Reflected Light

Introduction

In 1979, Hartwig and Kiihl reported a trait in PI 159925 soybean that delayed flowering under short-day conditions. The trait has been incorporated into temperate soybean germplasm and termed "long-juvenile" (Hinson, 1989). In addition to the photoperiodic response, Tomkins et al. (1994) reported that the long-juvenile (LJ) trait may also be influenced by red (R) and far red (FR) light quality components based on research conducted in a growth chamber environment. Natural sunlight, however, also contains a blue (B) light component that modulates the R/FR phytochrome response. One approach to manipulating the spectral characteristics of natural sunlight is the use of various reflective surfaces (Decoteau et al., 1988; Hunt et al., 1989; Decoteau et al., 1990). Therefore, a greenhouse study was performed using LJ soybean near-isoline pairs grown under natural light conditions with contrasting reflective light treatments to provide different spectral balances of R, FR, and B light.

Materials and Methods

Three LJ near-isoline pairs (F85-1107/1108, F85-372/369-1, and F85-1020/1027) were established in 355 ml styrofoam cups containing soil in which soybeans had been grown previously. The soil was a Riverview loam (fine-loamy, mixed, thermic Fluventic Dystrochrepts) which did not receive supplemental fertilizer because soil test results indicated that fertility levels were near optimal for soybean growth. After emergence, plants were thinned to one plant per cup and placed equidistantly 20 cm apart on white and black plastic reflective mulches in the greenhouse under short-day conditions on 27 February (Clemson, SC; lat 34° N). Spectral measurements were taken 40 cm (incoming) and 20 cm (reflected) above the reflective surface in the center of each mulch treatment about solar noon using a LI-COR 1800 spectroradiometer (LI-COR, Lincoln, Neb.). Soil temperature (27°C) about solar noon was similar between treatments and not considered to be a factor in altering plant growth. Nondestructive measurements of plant height (cotyledonary node to terminal bud) and number of main stem nodes (not including the cotyledonary node) were taken 38 days after emergence. At first flower (R1 growth stage), individual plants were harvested and plant height, number of main stem nodes, leaf area (LI-COR LI 3100 leaf area meter), shoot fresh weight, and days to R1 from emergence were determined.

The experimental design was a completely randomized design with two factors (genotype and light quality treatment) and five replications per

isoline/treatment combination. Data were analyzed with SAS (Cary, NC) using general linear model procedures. Means were separated using the LSD method at the 0.05 probability level.

Results and discussion:

Spectral characteristics indicated that the white and black treatment surfaces differed markedly in the amount and quality of light reflected (Table 1). Compared to full sun, the greenhouse environment provided an average 20% decrease in incoming photosynthetic photon flux (PPF). The white and black surfaces reflected 23% and 4% of the incoming PPF, respectively. Reflected B light was 23% and 3% of incoming B light for white and black surfaces, respectively. The white surface reflected a 9% higher R/FR ratio compared to the black surface. Spectral characteristics for the reflective treatments were in good agreement with those reported in previous studies (Decoteau et al., 1988; Hunt et al., 1989).

Morphological characteristics evaluated at 38 days after emergence showed marked differences between reflection treatments (Table 2). Average plant height and internode length were both 27% greater for the black vs. the white treatments. Previous reports have associated low B light levels and reduced R/FR ratios with increased plant height and longer internodes (Ballare et al., 1987; Hunt et al., 1989; Ballare et al., 1991). Between reflective treatments, the magnitude of plant height and internode length effects were greater for the LJ compared with the normal-juvenile (NJ) isolines (Table 2). Within the white treatment, average NJ plant height and internode length was 24% and 21% greater than LJ isolines, respectively. Within the black treatment, there was no difference between LJ and NJ isolines for plant height and internode length. On an individual isoline pair basis, NJ isolines consistently had greater plant heights compared to LJ isolines within the white treatment but, responses to the black treatment varied between pairs. Internode length of NJ isolines was longer for two of the isoline pairs within the white treatment and similar within all pairs for the black treatment.

Days to R1 was not influenced by different spectral distributions as each isoline gave similar photoperiodic responses between reflection treatments (data not shown). Average days to R1 were 62 and 44 days for the LJ and NJ, isolines respectively. Average plant height and internode length measured at R1 were increased 29% and 31% in response to the decrease in the R/FR ratio, respectively. Like the first set of observations, the magnitude of plant height and internode length effects were greater for the LJ compared to the NJ isolines between reflective treatments (Table 3). Plant heights at R1 were greater for the LJ isolines due the average 18 day delay in flowering conferred by the LJ trait that allowed for extended vegetative growth. Average leaf area and shoot fresh weight values were similar between reflective treatments (Table 4). For leaf area and shoot fresh weight responses, the magnitude of the effects were greater for two of the LJ isolines compared to NJ isolines between reflective treatments. Like plant height responses, LJ isolines showed increased levels of leaf area and shoot fresh weight compared to the NJ isolines due to the delay in flowering conferred by the LJ trait.

Table 1. Spectral characteristics of incoming and reflected light of white and black reflective surfaces in the greenhouse. Data taken at about solar noon.

Spectral source	PPF[a]	Blue[b]	R/FR[c]
		μmol m^{-2} sec^{-1}	
Full sun	1640	418	1.15
White (incoming)	1231	307	1.17
White (reflected)	282	71	1.19
Black (incoming)	1382	350	1.14
Black (reflected)	51	11	1.09

[a] PPF = photosynthetic photon flux; 400 to 700 nm.
[b] Blue; 400 to 500 nm.
[c] R/FR = red/far red; 660 to 670/730 to 740 nm.

Table 2. Plant height and internode length of soybean long-juvenile near-isoline pairs grown over white and black reflective surfaces. Data taken at 38 days after emergence.

	Plant height			Internode length		
Isoline	Black	White	Δ	Black	White	Δ
			mm $plant^{-1}$			
LJ isolines	107	76	31*	26	19	7*
NJ isolines	110	94	16*	27	23	4*
Δ	-3	-18*		-1	-4*	
F85-1108 (LJ)	115	80	35*	29	19	10*
F85-1107 (NJ)	127	115	12*	32	28	4*
Δ	-12*	-35*		-3	-9*	
F85-369-1 (LJ)	74	62	12*	19	16	3*
F85-372 (NJ)	87	74	13*	22	19	3*
Δ	-13*	-12*		-3	-3*	
F85-1027 (LJ)	131	87	44*	30	21	9*
F85-1020 (NJ)	116	94	22*	28	22	6*
Δ	15*	-7*		2	-1	

* Differences significant at the 0.05 level of probability.

Table 3. Plant height and internode length of soybean long-juvenile near-isoline pairs grown over white and black reflective surfaces. Data taken at first flower.

Isoline	Plant height			Internode length		
	Black	White	Δ	Black	White	Δ
	mm plant^{-1}					
LJ isolines	201	146	55*	26	20	6*
NJ isolines	128	110	18*	26	22	4*
Δ	73*	36*		0	2	
F85-1108 (LJ)	161	117	44*	27	21	6*
F85-1107 (NJ)	127	115	12*	32	27	5*
Δ	34*	2		-5*	-6*	
F85-369-1 (LJ)	212	159	53*	23	17	6*
F85-372 (NJ)	120	98	22*	21	17	4*
Δ	92*	61*		2	0	
F85-1027 (LJ)	231	162	69*	30	21	9*
F85-1020 (NJ)	137	116	21*	26	22	4*
Δ	94*	46*		4*	-1	

* Differences significant at the 0.05 level of probability.

Table 4. Leaf area and shoot fresh weight of soybean long-juvenile near-isoline pairs grown over white and black reflective surfaces. Data taken at first flower.

Isoline	Leaf area			Shoot fresh weight		
	Black	White	Δ	Black	White	Δ
	cm^{-2} plant^{-1}			mg plant^{-1}		
LJ isolines	202	183	19	534	494	40
NJ isolines	124	113	11	299	290	9
Δ	78*	70*		235*	204*	
F85-1108 (LJ)	151	119	32	393	315	78
F85-1107 (NJ)	100	94	6	240	244	-4
Δ	51*	25		153*	71	
F85-369-1 (LJ)	226	225	1	600	625	-25
F85-372 (NJ)	136	119	17	326*	293*	33
Δ	90*	106*		274*	332*	
F85-1027 (LJ)	229	204	25	608	542	66
F85-1020 (NJ)	136	127	9	331	333	-2
Δ	93*	77*		277*	209*	

* Differences significant at the 0.05 level of probability.

Conclusion

Reflective treatments did not affect time of flowering in response to the LJ trait. This may be due to the presence of B light which is thought to play an important role in determining daylength responses. If so, then the putative 'cryptochrome' action in response to B light between LJ and NJ genotypes may operate in the same manner. However, photomorphogenic responses as affected by the LJ trait varied between reflective treatments in this study as they did in previous research (Tomkins et al., 1994). Because R and FR photomorphogenic responses are modulated by phytochrome, our results suggest that phytochrome mediated growth responses differ between LJ and NJ soybean genotypes.

References

Ballare, C. L., J. J. Casal, and R. E. Kendrick. 1991. Responses of light-grown wild-type and long-hypocotyl mutant cucumber seedlings to natural and simulated shade light. Photochem. and Photobiol. 54:819-826.

Ballare, C. L., R. A. Sanchez, A. L. Scopel, J. J. Casal, and C. M. Ghersa. 1987. Early detection of neighbor plants by phytochrome perception of spectral changes in reflected sunlight.

Decoteau, D. R. , M. J. Kasperbauer, D. D. Daniels, and P. G. Hunt. 1988. Plastic mulch color effects on reflected light and tomato plant growth. Scientia Hort. 34:169-175.

Decoteau, D. R. , M. J. Kasperbauer, and P. G. Hunt. 1990. Bell pepper plant development over mulches of diverse colors. Hort. Sci. 25:460-462.

Hartwig, E. E., and A. S. Kiihl. 1979. Identification and utilization of a delayed flowering character in soybeans for short-day conditions. Field Crops Res. 2:145-151.

Hinson. K. 1989. Use of a long-juvenile trait in cultivar development. p. 983-987. *In* A. J.

Pascale (ed.) Proc. World Soybean Res. Conf. IV. Buenos Aires, Argentina. 5-9 Mar. 1989. Argentina Soybean Assoc., Buenos Aires.

Hunt, P. G., M. J. Kasperbauer, and T. A. Matheny. 1989. Soybean seedling growth responses to light reflected from different colored soil surfaces. Crop Sci. 29:130-133.

Tomkins, J. P., E. R. Shipe, and S. U. Wallace. 1994. Photomorphogenic influence of light quality on expression of the long-juvenile trait in soybean. Soybean Gen. Newsl. 21:261-265.

Jeff P. Tomkins

Dennis R. Decoteau

CLEMSON UNIVERSITY
Department of Agronomy
Clemson, South Carolina 29634

Relationships among Plant Characters in Soybean with Different Seed Size

Seed size, which has gained attention by virtue of increasing specialty market demand, has usually been studied from a seed production standpoint, mostly in relation with seed yield (Burris et al., 1973; Fontes and Ohlrogge, 1972; Johnson and Luedders, 1974; Smith and Camper, 1975). Genetic alteration of seed size did not seem to influence seed yield significantly (Smith and Camper, 1975), possibly due to compensation by other yield components. Correlations between seed size and other yield components have been reported (Johnson et al., 1955; Fontes and Ohlrogge 1972); however, pod width (Bravo et al., 1980) was the first plant characteristic reported as a practical indicator for seed size. Matsumoto and Umezaki (1987) reported plant architectural differences between small and large seeded cultivars, and they found a relationship between yield components and plant morphology. Genetic alteration of seed size will likely influence other morphological and physiological characters, and identification of these characters would provide direction for breeding programs aimed for large and small seeded cultivars. The objective of this research was to evaluate characters which are correlated with seed size using genotypes with different genetic backgrounds.

Materials and Methods

Eleven determinate soybean genotypes from maturity group V, VI, and VII, which have a wide range of seed sizes, were used in this experiment. The materials were planted on 15 June 1994 at Clemson's Simpson Experiment Research Station near Pendleton, SC, in a Chewacla silt loam (Fine-loamy, mixed, thermic Fluvaquentic Dystrochrepts) soil. Fertilizer was applied prior to planting at a rate of 0-36.7-0 kg ha^{-1} (N-P-K). The experimental design was a randomized complete block design with four replications. A plot consisted of four rows, 6m long and 0.96m apart. Approximately 200 seeds were sown in each row, and plants were thinned to a uniform population of 23 plants m^{-1} row^{-1} at 30 days after germination.

Dates of flowering (R1), beginning pod fill (R5), beginning maturity (R7), and full maturity (R8) (Fehr and Caviness, 1971) were recorded. At harvest maturity, row sections containing twenty consecutive plants were randomly selected and harvested from one of the two interior rows. Individual plants were measured for 100 seed weight, plant height, number of branches, nodes per main stem, nodes on branches, pods per main stem, pods on branches, seeds per plant, pod width, plant dry weight, and lodging. These data were used to calculate seeds per pod and harvest index. Seed yields were measured on two interior rows of each plot that had been trimmed to 4.2m for harvest. Pod width was

measured as described by Bravo et al. (1980). Seeds were air dried and adjusted to 13% moisture basis for yield comparisons. All data were subjected to analysis of variance and mean separation done according to LSD. Correlation analyses among yield and yield components were based on individual plot mean values.

Results and Discussion

The year of 1994 was characterized by having moderate growing conditions for soybeans with excessive moisture at the beginning of the growing season. There was approximately a 2.1 fold difference in 100 seed weight (seed size) and a 1.4 fold difference in seed yield among entries (Table 1).

Seed yield showed no significant correlation with number of seeds per plant, nor with pods per plant, which are normally considered as good indicators of seed yield (Table 2). Seeds per plant was negatively correlated with seed size. Figure 1 shows the relationship between seed size, seeds per plant, and seed yield. Calculated yields from twenty sample plants ranged from approximately 2000kg ha^{-1} to 5000kg ha^{-1} in all seed size groups, while showing a wide range of differences in seed size and seeds per plant. These observations indicate that seed size and seeds per plant were strongly compensating each other at every level of yield, and it may be unreasonable to expect to increase seed yield by modifying just one character. Most morphological characters that had significant positive correlations with seeds per plant showed negative correlations with seed size (Table 2). Seeds per plant was positively correlated with number of branches, number of nodes per plant, number of nodes on branches, and plant dry weight. These results indicate that obtaining sufficient fruiting sites, especially on branches, is critical to increase number of seeds. Seed size had a strong correlation with pod width (0.98). It was much higher than the observation reported by Bravo et al. (1980) (0.64). It may be due to the greater range in seed size of genotypes evaluated in this study.

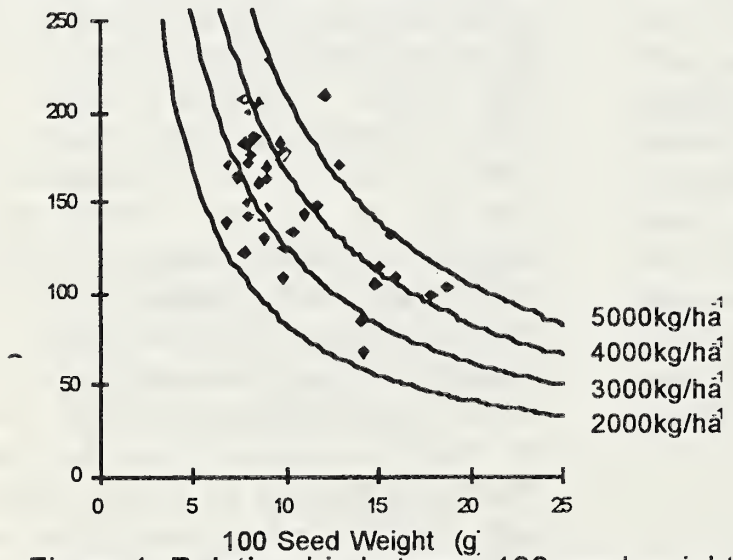


Figure 1. Relationship between 100 seed weight an number of seeds per plant in eleven genotypes.

Table 1. Average values of plant and seed characters in eleven genotypes.

	100 Seed Weight (g)	Seed Yield (kg ha-1)	Seeds / Plant	Seed Filling Duration (Days)	Maturity Date (Days)	Plant Height (cm)	Branches	Harvest Index (%)	Lodging (1-5)
SC92-68	7.6a	2073	180	45	143	86	4.0	42	2.5
SC92-64	7.9ab	1895	139	45	143	86	2.5	41	2.3
SC92-18	8.4abc	1930	135	41	139	113	5.3	44	5.0
PI518833	8.6abc	1777	139	40	118	70	3.3	45	3.8
SC92-63	8.6abc	2058	167	43	141	88	2.2	46	2.8
Pearl	9.1bc	2562	152	45	136	74	2.3	49	1.0
SC92-16	9.3bc	1937	188	41	140	115	5.5	44	5.0
Camp	9.3c	2197	146	43	125	51	2.5	55	1.0
Doles	12.0d	2298	166	52	140	91	3.7	49	3.5
NTC92-60	15.7e	2070	100	47	131	78	3.0	49	3.8
Stonewall	16.0e	2194	101	47	131	94	2.3	46	3.8

Means followed by the same letter do not differ at the 0.05 probability level.

Table 2. Phenotypic correlations between characters in eleven soybean genotypes with different seed size.

	Seed Yield	100 Seed Weight	Seeds / Plant	Veg. Duration	Rep. Duration	% Rep. Duration	S. F. Duration	% S. F. Duration
100 Seed Weight	0.43**	1						
Seeds / Plant	-0.11	-0.59***	1					
Vegetative Duration	-0.25	-0.45**	0.55***	1				
Rep. Duration	0.47**	0.32*	-0.12	-0.09	1			
% Rep. Duration	0.42**	0.52***	-0.53***	-0.90***	0.50***	1		
S. F. Duration	0.48***	0.55***	-0.23	-0.18	0.83***	0.51***	1	
% S. F. Duration	0.41**	0.68***	-0.54***	-0.79***	0.41**	0.85***	0.71***	1
Pod Width	0.50**	0.96***	-0.58***	-0.43**	0.31*	0.50***	0.57***	0.69***
Pods / Plant	-0.24	-0.53***	0.94***	0.50***	-0.20	-0.53***	-0.23	-0.47**
Seeds / Pod	0.39**	-0.15	0.19	0.13	0.22	-0.02	0.01	-0.17
Harvest Index	0.46**	0.33*	-0.11	-0.60***	0.30*	0.66***	0.22	0.47**
Plant Height	-0.16	0.02	0.32*	0.73***	-0.10	-0.69***	0.01	-0.43**
Branches	-0.22	-0.15	0.61***	0.51***	-0.35*	-0.60***	-0.17	-0.35*
Nodes / Plant	-0.32*	-0.52***	0.79***	0.86***	-0.10	-0.80***	-0.17	-0.70***
Nodes / Main Stem	-0.17	-0.52***	0.41***	0.70***	0.29*	-0.49***	0.07	-0.53***
Nodes / Branches	-0.32*	-0.40**	0.77***	0.73***	-0.26	-0.75***	-0.24	-0.61***
% Nodes-Main Stem	0.30*	0.29	-0.73***	-0.50***	0.34*	0.59***	0.25	0.43**
Plant dry Weight	0.01	0.17	0.52***	0.50***	-0.00	-0.45**	0.17	-0.18
Lodging	-0.18	0.28	0.18	0.32*	-0.35*	-0.44**	-0.12	-0.17

*,**,*** Significant at the P = 0.05, 0.01, and 0.001 level of probability respectively.

Length of vegetative, reproductive, and seed filling period influenced seed yield, yield components, and plant architecture. Seed yield had the strongest correlation with seed filling duration, whereas seed size had the strongest correlation with relative length of seed filling duration to total growth period (% seed filling duration). Seeds per plant was strongly correlated with vegetative duration as well as relative length of reproductive duration (% reproductive duration). Vegetative and % reproductive duration also had strong correlations with most parameters of vegetative growth, such as plant height, number of branches, number of nodes, and plant dry weight. These results indicate that longer vegetative duration is important to increase vegetative growth and total number of seeds produced on plants.

Results of this experiment suggest that length of vegetative, reproductive, and seed filling periods are fundamental characters to consider when breeding for seed size and seed yield in determinate soybeans. The consistent trend observed between vegetative and reproductive duration, plant architecture, and yield components agreed with the findings reported by Matsumoto and Umezaki (1987). Considering the high heritability estimates reported for flowering date and maturity (Burton, 1987), it is conceivable to think that vegetative and reproductive duration strongly influences the balance between yield components and supporting plant architecture.

Results presented here suggest the following hypothesis regarding relationships between seed size and vegetative, reproductive, and seed filling duration. Small seeded genotypes require a large number of fruiting sites to support large number of seeds. Increase of fruiting sites requires more vegetative growth which leads to longer vegetative duration and likely, shorter reproductive duration. On the other hand, large seeded genotypes require a small number of fruiting sites to accumulate photosynthate in a minimum number of seeds. Maintaining minimum fruiting sites requires limited vegetative growth, which leads to shorter vegetative duration and likely, longer reproductive duration. Therefore, the balance between vegetative, reproductive, and seed filling duration within a given total growth period is critical in breeding for seed size and high seed yield. If this hypothesis is proven to be true, breeders may find the length of growth periods as useful indicators when selecting for seed size.

References

Bravo, J. A., W. R. Fehr, and S. Rodriguez de Cianzio. 1980. Use of pod width for indirect selection of seed weight in soybeans. Crop Sci. 20:507-510.

Burris J. S., A. H. Wahab, and O. T. Edje. 1973. Effect of seed size on seedling performance in soybeans. II. Seedling growth and photosynthesis and field performance. Crop Sci. 13:207-210.

Burton J. W. 1987. Quantitative genetics: Results relevant to soybean breeding. In J. R. Wilcox (ed.) Soybeans: Improvement, production, and use. 2nd ed. Agronomy 16:211-247.

Fehr, W. R., and C. R. Caviness. 1977. Stages of soybean development.

Iowa State Univ., Coop. Ext. Serv. Spec. Rep. 80.

Fontes L. A. N. and A. J. Ohlrogge. 1972. Influence of seed size and population on yield and other characteristics of soybean. Agron. J. 64:833-836

Johnson, H. W., H. F. Robinson, and R. E. Comstock. 1955. Genotypic and phenotypic correlations in soybeans and their implications in selection. Agron, J. 47:477-483.

Johnson, D. R. and V. D. Luedders. 1974. Effect of planted seed size on emergence and yield of soybeans. Agron. J. 66:117-118

Matsumoto, s., and T. Umezaki. 1987. Grouping of soybean varieties on the basis of seed number, 100 seed-weight ratio. Japan Jour. Crop Sci. 56:177-183.

Smith, T. J., and H. M. Camper, Jr. 1975. Effects of seed size on soybean performance. Agron. J. 67:681-684.

Kenji Saka

Emerson R. Shipe

Susan U. Wallace

THE UNIVERSITY OF TENNESSEE
Plant Molecular Genetics and Center for Legume Research and
Department of Plant and Soil Science
Knoxville, TN 37901-1071

Application of DAF Technology to Find Potential Markers Associated with Resistance to Soybean Cyst Nematode, Race 3

Introduction

Soybean cyst nematode (SCN, *Heterodera glycines* Ichimohe) is the leading pest causing economic losses of soybean yields in the USA. It was first found in North Carolina in 1954 (Winstead et al., 1955); and has subsequently been reported in most soybean producing states of the USA. It has been estimated that the combined yield losses from SCN in 28 soybean producing states during the years 1988-1991 was approximately $800 million per year (Doupnik, 1993; Sciumbato, 1993). Chemical control of SCN has been widely used, but may cause environmental pollution when used extensively. Crop rotation is another effective way to manage nematode populations in infested soil; however, cultivation for one season of a susceptible cultivar can result in resurgence of the nematode population. The development of resistant cultivars is a high priority in many public and private soybean breeding programs, and the use of such cultivars is an effective approach to reduce soil populations and damage from SCN. Many existing cultivars with resistance suffer from yield depression. The techniques to identify resistant versus susceptible genotypes is time- and labor -consuming and imprecise. Identification and characterization of new resistance genes in soybean by molecular methods will play an important role in introgressing these genes into new cultivars (Webb et al., 1995; Concibido et al., 1994).

We are using DNA amplification fingerprinting (DAF) to identify resistance gene(s) associated with Race 3 of SCN in soybean.

Materials and Methods

Two soybean genotypes are used in this study. One is cultivar Essex, susceptible, and the other DPS3589, a line resistant to Race 3 from Delta & Pine Land Co. DPS3589 was chosen because its resistance to Race 3 appears to be conditioned by a single recessive gene (i.e., similar, but not necessarily identical to rhg1 or rhg2, see Rao-Arelli et al., 1992).

Plant DNA was isolated using established procedures. DNA amplification was done as outlined by Bassam et al. (1991) and Caetano-Anollés and Gresshoff (1994). We utilized a single arbitrary primer method, DAF, coupled with high resolution polyacrylamide gel electrophoresis and silver-staining, to construct a catalogue of polymorphisms between the parental lines, permitting the future analysis of F2/F3 bulks using bulked segregant analysis (Michelmore et al., 1991)

Several DAF primers including 20 octamers and 86 mini-hairpins (mhp) (Caetano-Anollés and Gresshoff, 1994) were screened for their ability to detect DNA polymorphisms among Essex and DPS3589.

Results and Discussion

The average primer gave 90-120 bands on a 60 cm long PAGE gel. However, our analysis was done with Mini-Protean II gels (8 cm x 10 cm), on which about 30 to 40 bands are resolved (on average per primer). Of these, many were faint and were not scored. A total of 3400+ data points were obtained (Table 1). Out of 106 primers (86 mini-hairpins and 20 octamers) used, 41 (35 mini-hairpins and 6 octamers) showed polymorphisms (Table 1). Sixty-five polymorphic bands (53 for mini-hairpins and 12 for octamers) were observed; and percent polymorphic bands for mini-hairpin primers and octamers were 1.96 and 1.71, respectively (Table 1).

Table 2 presents the polymorphisms between DPS3589 and Essex detected by DAF primers and their characteristics. Of the 65 polymorphic bands observed, 33 belonged to DPS3589 and 32 were seen in Essex. Most primers generated a single polymorphic band, however, some primers such as mini-hairpin primers A-47, B-17, C-3, C-4, C-13, C-21, C-24, D-5, D-6, and octamers 8-9, 8-13, and 8-47 produced 2, 3 or even 4 polymorphic bands. This higher resolution of mini-hairpin primers stems from the relatively short 3' extension of 3 nucleotides.

The mini-hairpin primers belong to complete sets of 64, which differ in the hairpin sequence. Thus mhpA1 has the same 3' nucleotides (3) as mhpB1. We note that both primers detected polymorphisms of the same molecular weight and in the same genotype. However, several other pairwise comparisons showed that the mini-hairpin itself influences the detection of the amplification polymorphism. For example, mhpC1 also detected a polymorphism, but of different size than A1 and B1. Additionally, we note that C1 may detect a codominant polymorphism as noted by the reciprocal absence/presence of two bands. Of interest, this was also detected for some other primers. The genetic assignment of the mode of inheritance will require F2 segregant analysis.

Crosses have been made between DPS3589 and Essex, and their F1 progeny have been screened for reaction to Race 3 of SCN. Approximately 2000 F2 generation plants have also been visually scored (scale 1 to 5) for reaction to Race 3. In addition, a subset of 315 of the F2 plants was chosen and female cysts on the roots were removed and counted. Extreme plants with highest and lowest nematode counts were chosen (Table 3) and DNA was extracted from each plant. Resistant and susceptible F2 DNA bulks were made by mixing equal amounts of DNA of each of the 11 plants with lowest (R) and each of the 11 plants with highest number of nematodes (S), respectively. Our ultimate goal is to do bulked segregant analysis (Michelmore et al., 1991) to find the genetic location of the SCN resistance locus responsible for the phenotype of DPS3589.

Table 1. DAF polymorphisms found with mini-hairpin and octamer primers

Primer type	# Primers tested	# Bands generated	# Polymorphic primers	# Polymorphic bands	% Polymorphic bands
Mini-hairpins	86	2700	35	53	1.96
Octamers	20	700	6	12	1.71
Total	106	3400	41	65	1.91

Table 2. The polymorphisms between DPS3589 and Essex detected by DAF primers and their characteristics

Primer code		Primer sequence	DPS3589	Essex	MW (in bp)
Mini-hairpin	A1	GCGAAAGC-CCC	-	+	990
"	A2	-CCT	+	-	390
"	A3	-CCG	-	+	320
"	A5	-CTC	+	-	480
"	A6	-CTT	+	-	240
"	A9	-CGC	+	-	440
"	A13	-CAC	+	-	450
"	A23	-TTG	+	-	500
"	A25	-TGC	-	+	160
"	A27	-TGG	+	-	340*
"	A27	-TGG	-	+	510*
"	A28	-TGA	+	-	440
"	A47	-GAG	+	-	720, 850
"	B1	GCGACAGC-CCC	-	+	990
"	B2	-CCT	-	+	900
"	B3	-CCG	-	+	170
"	B6	-CTT	+	-	480
"	B17	-TCC	-	+	270, 380
"	B19	-TCG	+	-	430*
"	B19	-TCG	-	+	440*
"	B20	-TCA	-	+	350
"	B22	-TTT	-	+	400
"	B25	-TGC	+	-	500
"	C1	GCGAGAGC-CCC	-	+	240*
"	C1	-CCC	+	-	260*
"	C2	-CCT	-	+	320
"	C3	-CCG	+	-	280*, 320*
"	C3	-CCG	-	+	310*
"	C4	-CCA	+	-	250, 290
"	C5	-CTC	+	-	390
"	C13	-CAC	+	-	290, 500
"	C16	-CAA	-	+	320
"	C21	-TTC	-	+	350, 400
"	C24	-TTA	+	-	650*
"	C24	-TTA	-	+	270*, 310*
"	C25	-TGC	+	-	270
"	C39	-GTG	+	-	300*
"	C39	-GTG	-	+	465*
"	D2	GCGATAGC-CCT	+	-	370
"	D5	-CTC	+	-	150, 180, 330
"	D6	-CTT	+	-	240, 320, 360, 380
Octamer	8-9	GTTACGCC	-	+	225, 235, 560
	8-10	GTATCGCC	-	+	220
	8-12	GTAACCCC	-	+	500
	8-13	GTAACGGC	-	+	120, 150, 290, 600
	8-16	ACCCAACC	+	-	500
	8-47	GCCCGCCC	-	+	220, 290

*possible codominant alleles

Table 3. Resistant and susceptible F2 plants used to make R & S bulks, and their cyst count

Resistant F2 plants (R)		Susceptible F2 plants (S)	
Cross DxE 11-3*:		Cross DxE 3-2:	
Plant #	No. of cysts	Plant #	No. of cysts
1	9	4	358
56	13	8	361
77	11	9	373
112	9	12	469
115	10	15	363
116	4	28	450
117	13	39	407
118	13	49	398
136	3	52	459
		65	615
Cross DxE 3-3:		**Cross DxE 3-3:**	
22	9	15	392
35	13		

* D=DPS3589, E=Essex Note: resistance in DPS3589 is not absolute

References

Bassam, B. J., Caetano-Anollés, G. , and Gresshoff, P. M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry 196:80-83.

Caetano-Anollés, G., and Gresshoff, P. M. 1994. DNA amplification fingerprinting using arbitrary mini-hairpin oligonucleotide primers. Bio/Technology 12:619-623.

Concibido, V. C., Denny, R. L., Boutin, S. R., Hautea, R., Orf, J. H., and Young, N. D. 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Science 34:240-246.

Doupnik, B. Jr. 1993. Soybean production and disease loss estimates for north central United States from 1989-1991. Plant Dis. 77:1170-1171.

Michelmore, R. W., Paran, I., and Kesseli, R. V. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc. Nat. Acad. Sci. (USA) 88:9828-9832.

Rao-Arelli, A. P., Anand, S. C., and Wrather, J. A. 1992. Soybean resistance to soybean cyst nematode Race 3 is conditioned by an additional dominant gene. Crop. Science 32 (4):862-864.

Sciumbato, G. L. 1993. Soybean disease loss estimates for the southern United States during 1988-1991. Plant Dis. 77 (9):954-956.

Webb, D. M., Baltazar, B. M., Rao-Arelli, A. P., Schupp, J., Clayton, K., Keim, P., and Beavis, W. D. 1995. Genetic mapping of soybean cyst nematode Race-3 resistance loci in the soybean PI437.654. Theoret. Appl. Genet. 91:574-581.

Winstead, N. N., Skotland, C. B., and Sasser, J. N. 1955. Soybean cyst nematode in North Carolina. Plant Disease Rept. 39:9-11.

B. Yazdi-Samadi P. M. Gresshoff
L. Young F. L. Allen

UNIVERSITY OF TENNESSEE
Plant Molecular Genetics
Institute of Agriculture and Center for Legume Research
Knoxville, TN 37901-1071

Identification of a Linkage-Group-H-Terminal ASAP Marker Linked to the Supernodulation Locus in Soybean using Bulked Segregant Analysis

Supernodulation is a trait caused by mutation in nitrate-tolerant-symbiosis (*nts*) genes that control the number and mass of nodules formed on the roots of legumes (Caetano-Anollés and Gresshoff, 1991). A chemical mutagenesis program in soybean (*Glycine max* L. Merr.) identified a number of mutants with increased nodulation in the absence or presence of fixed nitrogen (Carroll et al., 1985a,b). A total of twelve mutant alleles have been characterized by complementation analysis and define the supernodulation *nts* locus (Delves et al., 1988). The *nts* mutation was found to be recessive and unlinked to the nonnodulation *nod49* and *nod139* loci, and its expression epistatically suppressed in double mutants homozygous for the *nts* and *nod* alleles (Mathews et al., 1990). Despite considerable effort, no association of each mutation to a biochemical step or cloned nodulin gene has yet been identified.

Pursuing a map-based cloning approach to isolate the *nts* gene, F_2 populations from crosses between *G. soja* (carrying the wild-type *nts* allele) and supernodulating lines nts382 and nts1007 were analyzed for co-segregation of RFLP markers with the mutant gene. Screening of a set of RFLP molecular markers showed that marker pUTG-132a (derived from pA-132 in linkage group H) was tightly linked to the *nts* locus (Landau-Ellis et al., 1991). This RFLP marker was converted into a PCR-based STS and used to screen a large F_2 segregating population (representing 330 plants) (Kolchinski et al., 1996). The STS was placed only 0.4-1.1 cM away from *nts* and ordered with respect to regional markers, was unusually conserved as judged by sequence analysis of the STS obtained from other soybean varieties (Peking, Enrei, Minsoy, Noir 1 and DPS3589), and was detected in a single chromosomal location by fluorescent *in situ* hybridization (FISH) to soybean metaphase chromosomes (P. Keim, unpublished).

In order to construct a high density map around the *nts* region, DNA pools from segregants homozygous for pUTG-132a and flanking markers pA-381 and pA-36 were analyzed by genome scanning with 181 unstructured primers (Kolchinsky et al. 1996). While the strategy maximized the window for detection of potential polymorphisms, only two DNA polymorphisms were identified. They were subsequently cloned and converted into SCARs. Only one of them (pCR54-L) was truly polymorphic and mapped 15.4 cM from marker pA-36, outside the cluster.

Because overall results suggest that the *nts* region is unusually conserved, fingerprint tailoring strategies were used to increase detection of polymorphic DNA in the *nts* region. Pre-digestion of template DNA with three endonucleases prior to amplification with octamer primers in tecMAAP, distinguished EMS-induced mutant nts382 from its wild-type parent and resulted in the detection of 42 polymorphisms, 14 of which segregated at 100% with the supernodulation phenotype in *G. soja* x *G. max* mutant derived F_2 populations (Caetano-Anollés et al., 1993). In contrast, a second screening of phenotypic pools of the *nts* locus in bulked segregant analysis (BSA; Michelmore et al., 1991) by amplification with 120 octamers did not produce additional polymorphisms. We therefore used arbitrary signatures from amplification profiles (ASAP) (Caetano-Anollés and Gresshoff, 1996) to identify markers linked to *nts* in BSA. ASAP analysis produced a linkage-group-H-terminal marker linked to *nts*.

Materials and Methods

Glycine soja PI468.397 was crossed with *G. max* nts382, a supernodulating EMS-induced mutant of soybean cv. Bragg, to produce F_2 and F_3 segregating populations. DNA was extracted using established protocols (cf. Caetano-Anollés et al. 1991), and concentrations measured by fluorescent enhancement of dye H33258 (Hoeffer, San Francisco, CA). DAF reactions were in a total volume of 20 µl as described (Caetano-Anollés and Gresshoff 1994) and contained 3 µM primer, 200 µM of each deoxynucleoside triphosphates, 0.3 units/µl AmpliTaq Stoffel fragment DNA polymerase (Perkin Elmer-Cetus, Norwalk, CT), 0.1 to 5 ng/µl of template DNA, and 1.5 mM $MgCl_2$ and Stoffel buffer (10 mM KCl, 10 mM Tris-HCl ; pH 8.3) when using standard primers, or 4 mM $MgSO_4$ and TTNK10 buffer (10 mM KCl, 4 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 20 mM Tris·HCl; pH 8.3)(Caetano-Anollés et al., 1994) when using mini-hairpin primers. The mixture was amplified in a recirculating hot-air thermocycler for 35 cycles of 30 s at 96°C, 30 s at 30°C, and 30 s at 72°C. ASAP reactions contained 9 µM primer and 0.1 ng/µl template concentrations. ASAP reactions with SSR primers were amplified for 35 cycles of 30 s at 96°C, 60 s at 55°C, and 30 s at 72°C and using 3µM primer concentrations. Amplification products were separated in polyester-backed 5% polyacrylamide-7M urea slab mini-gels (Caetano-Anollés and Gresshoff, 1994). Wells were generally loaded with 3 µl of a 1:5 dilution of each amplification mixed with 3 µl of loading buffer (10 M urea, 0.08% xylene cyanol FF), and electrophoresis run at 120 V for approximately 75 min. ASAP reactions using SSR primers were diluted 1:50 prior to electrophoresis. DNA was detected by silver staining (Bassam and Caetano-Anollés, 1993). Backed gels preserved by drying at room temperature. Data obtained from segregating populations were analyzed using Mapmaker version 1.0 for the Macintosh (E.I. DuPont de Nemours Inc., Wilmington, VA).

Results and Discussion

Genome scanning strategies use arbitrary oligodeoxinucleotide primers to target discrete sites characteristic of a nucleic acid template and generate molecular markers for genome mapping and general fingerprinting applications (Caetano-Anollés, 1994). DAF , one of such strategies, produces nucleic acid profiles with high multiplex ratios using standard primers of 5-8 nt in length (Caetano-Anollés et al., 1991) or

extraordinarily stable mini-hairpin primers harboring a "core" arbitrary sequence at the 3' terminus (Caetano-Anollés and Gresshoff, 1994). Mini-hairpin primers can be used to fingerprint a wide variety of templates, ranging from plasmids and PCR products to complex plant and animal genomes, and detect polymorphic DNA with high efficiency. In this study, mini-hairpin primers with short arbitrary cores, standard octamers, and primers complementary to simple sequence repeats (SSR) were used in ASAP analysis of the *nts* region. ASAP is as a novel "fingerprint tailoring" strategy capable of distinguishing closely related organisms (Caetano-Anollés and Gresshoff, 1996). ASAP produces specific signatures by re-amplifying DAF profiles with mini-hairpin or standard arbitrary primers, and therefore generating "fingerprints of fingerprints" that provide additional scanning of primary sequence.

Reproducible ASAP profiles were produced upon optimization of amplification reaction parameters, mainly primer and template concentration; reliably profiles were obtained both from a same or replicated DAF amplification or from different DNA extractions. Mini-hairpin decamers and standard octamers required at least 6 µM or 9 µM primer concentrations, respectively, and template concentration within the range 0.001-1 ng/µl.

BSA was used to generate markers associated with the supernodulation trait. Pooled DNA samples from individuals in a segregating population that expressed or failed to express the phenotype were analyzed using DAF or ASAP analysis with standard octamers or mini-hairpin primers. DAF screening with 103 octamers failed to produce polymorphic markers. In contrast, amplification of bulks with 64 mini-hairpin decamers produced two polymorphic loci. Similarly, ASAP with just few mini-hairpin primers identified several polymorphisms between the bulks. About 8 putative markers were detected with 10 ASAP produced with 7 octamers and mini-hairpin decamers from DAF profiles generated with 2 octamer primers. The different combinations tested (10 out of 81 possible ASAP) always rendered distinct fingerprints. Results indicate that ASAP detected polymorphic DNA between the bulks at 50-fold higher levels than did DAF with mini-hairpin primers. Bonafide and tightly linked markers are now being converted into SCARs.

Primers can derive from known sequences representing dispersed DNA or structural chromosomal domains. Sequences in repetitive DNA, such as simple sequence repeats (SSR) present in microsatellites, are ubiquitous and highly polymorphic, and can be successfully targeted with motif-encoded primers. Several strategies based on the direct but arbitrary amplification of microsatellites are now available (Meyer et al., 1993; Perring et al., 1993; Wu et al., 1994; Zietkiewicz et al., 1994), providing markers that are usually co-dominant and express many allelic variants. However, the amplification of the SSR motif coexits with the amplification of unrelated arbitrary sequences and the true nature of microsatellite markers requires confirmation by hybridization to an SSR oligonucleotide (Weising et al., 1995). Relatively simple ASAP sequence signatures were generated by reamplification of DAF profiles with degenerate 5' anchored SSR primers, such as NN(AG)6, NN(CT)6 and NN(TG)6. The SSR primers were anchored at their 5' termini with ambiguous (degenerate) bases because primers had to target about 10-15 kb of DAF amplified sequence that contained only few SSR annealing sites, and were used in stringent

amplification conditions to avoid mismatch priming. Under these circumstances, the amplification of arbitrary sequences unrelated to the SSR motif was considerably decreased, and only few abundant amplification products were produced in about 50% of primer combinations tested. A microsatellite ASAP polymorphism (185 bp) between BSA pools produced using SSR primer NN(AG)6, segregated as a Mendelian marker and mapped 40 cM from marker pA-381, away from the *nts* locus (Fig. 1). This ASAP marker appears terminally located within linkage group H and may be close from the telomeric chromosomal region.

Our results show that BSA and DAF-derived genome scanning techniques can be successfully coupled in our search for *nts*-associated amplification markers with which to screen soybean YAC and BAC libraries (under construction) and anchor initial contigs. Despite the extremely conserved nature of the *nts* region (Kolchinsky et al., 1996), this coupling produced a number of putatively linked markers and one terminal ASAP marker distal to the *nts* region.

References

Bassam, B. J., G. Caetano-Anollés. 1993. Silver staining of DNA in polyacrylamide gels. Appl. Biochem. Biotechnol. 42:181-188.

Caetano-Anollés, G. 1994. MAAP: a versatile and universal tool for genome analysis. Plant Mol. Biol. 25:1011-1026.

Caetano-Anollés, G., P. M. Gresshoff. 1991. Plant genetic control of nodulation. Ann. Rev. Microbiol. 45:345-382.

Caetano-Anollés, G., P. M. Gresshoff. 1994. DNA amplification fingerprinting of plant genomes. Meth. Mol. Cell. Biol. 5:62-70.

Caetano-Anollés, G., P. M. Gresshoff. 1994. DNA amplification fingerprinting using arbitrary mini-hairpin oligonucleotide primers. Bio Technology 12:619-623.

Caetano-Anollés, G., P. M. Gresshoff. 1996. Generation of sequence signatures from DNA amplification fingerprints with mini-hairpin and microsatellite primers. Bio-techniques, in press.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1991. DNA amplification fingerprinting using short arbitrary oligonucleotide primers. Bio Technology 9:553-557.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1993. Enhanced detection of polymorphic DNA by multiple arbitrary amplicon profiling of endonuclease digested DNA: Identification of markers linked to the supernodulation locus in soybean. Mol. Gen. Genet. 241:57-64.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1994. Buffer components tailor DNA amplification with arbitrary primers. PCR Methods Applic. 4:59-61.

Carroll, B. J., D. L. McNeill, P. M. Gresshoff. 1995a. A supernodulation and nitrate tolerant symbiotic (*nts*) soybean mutant. Plant Physiol. 78:34-40.

Carroll, B. J., D. L. McNeill, P. M. Gresshoff. 1995b. Isolation and properties of soybean mutants which nodulate in the presence of high nitrate concentrations. Proc. Natl. Acad. Sci. USA 82:4162-4166.

Delves, A. C., B. J. Carroll, P. M. Gresshoff. 1988. Genetic analysis and complementation studies on a number of mutant supernodulating soybeans. J. Genet. 67:1-8.

Landau-Ellis, D., S. Angermüller, R. Shoemaker, P. M. Gresshoff. 1991. The genetic locus controlling supernodulation co-segregates tightly with a cloned molecular marker. Mol. Gen. Genet. 228:221-226.

Kolchinsky, A. K., D. Landau-Ellis, and P. M. Gresshoff. 1996. Molecular analysis of a polymorphic DNA region linked to the supernodulation (*nts*) locus of soybean. Mns. submitted.

Mathews, A., B. J. Carroll, P. M. Gresshoff. 1990. The genetic interaction between non-nodulation and supernodulation in soybean: an example of developmental epistasis. Theor. App. Genet. 79:125-130.

Meyer, W., T. G. Mitchel, E. Z. Freedman, R. Vilgalys. 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. J. Clin. Microbiol. 31:2274-2280.

Michelmore, R. W., I. Paran, R. V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, T. S. Bellows. 1993. Identification of whitefly species by genomic and behavioral studies. Science 259:74-77.

Weising, K., R. G. Atkinson, R. C. Gardner. 1995. Genomic fingerprinting by microsatellite-primed PCR: A critical evaluation. PCR Methods Applic. 4:249-255.

Wu, K, R. Jones, L. Danneberger, P. A. Scolnik. 1994. Detection of microsatellite polymorphisms without cloning. Nucleic Acids Res. 22:3257-3258.

Zietkiewicz, E., A. Rafalski, D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176-183.

Gustavo Caetano-Anollés

Peter M. Gresshoff

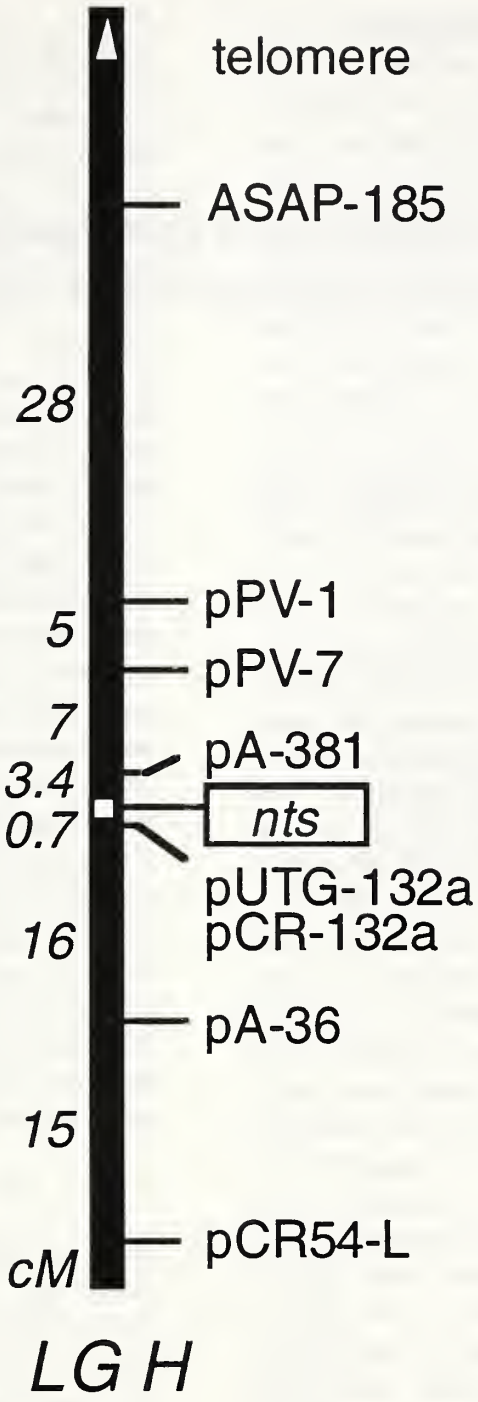


Figure 1. Regional map around the *nts* region. Markers are within linkage group H of the USDA-ARS (Iowa) RFLP map of soybean.pCR54-L is a SCAR marker and pPV-1 and pPV-7 are random genomic clones from *Phaseolus vulgaris*. The arrow shows proximity to the telomeric region. The ASAP marker (ASAP-185) appears terminal to this linkage group and the *nts* region.

VIRGINIA STATE UNIVERSITY
P. O. Box 9289
Petersburg, VA 23806

Planting Date Influence on Vegetable-Type Soybean Green Pod Yield, Individual and Total Sugars

Introduction

Soybean (*Glycine max* (L.) Merr.) with Japanese pedigree and large seeded is termed as vegetable -type soybean and it can be used for different food products. When harvested at green pod (R6-R7) stage, the pods can be either shelled to be used in salad like field peas or boiled to serve with pods. When harvested at dry seed stage it could be used for various soyfood products. The direct use of soybean holds promise because its protein content is twice of the other food legumes. Percentage oil, protein, fatty acids composition reach nearly maximum values at R6-R7 growth stages (Rubel et al., 1972). Generally, the pods and seeds of vegetable-type soybean are larger and easier to shell. The seed coat is loser and cracks more easily which probably accounts for their increased cookability compared to the grain-type. It has better flavor and palatability after cooking than the grain-type. Development of soybean as vegetable-type crop use is another way to further increase the versatility of soybean. The objective of this study was to determine planting dates effect on green pod yield and sugar content of vegetable-type soybean.

Materials and methods

The experiment was conducted in randomized complete block design (RCBD) arranged in split-plot treatment with four replications. The plot size was 3.66 m long with four-rows at 70 cm spacing between rows. Ten genotypes were planted at a rate of 23 seeds/meter at three planting dates (early-May, mid-May, and late-May) and three years. Each genotype was evaluated at R6-R7 growth stages by harvesting the two center-rows of each four-row plot. The harvested materials were put into plastic bags and brought to the laboratory. Pods were removed by hand from the harvested materials and weighed. Pods samples were taken and immature seeds were removed. The total sugar and individual sugars were determined using High Performance Liquid Chromatography (HPLC). The data were initially analyzed as RCBD in split-plot treatment combined over years. However, since significant year x treatment interaction occurred for several variables, data were analyzed separately for each year.

Results and Discussions

No significant differences was observed for Green pod yield among the three planting dates during the 1992 and 1994 growing seasons (Table 1), however, significant difference was observed in 1993. Early planting date had significantly higher yield than the mid-May or late May planting dates. Comparing the three year studies, the mean yield of 1992 was three times higher than the 1993 and about four-times than 1994 mean yield.

Significant planting date x genotype interaction was observed for green pod yield (Table 2). This indicates that performance of genotypes differ from one planting date to another. At mid May planting PI 417.310, Ware, and AGS 129 produced higher yield than the rest of genotypes tested. But the yield of AGS 129 was reduced by 17% and 25% when planted early and late, respectively. Similar response was observed by Ware, its yield was reduced by 30% when planted early, and by 18% when planted late and the yield of PI 417.310 was also reduced by 21% and 41%, respectively. On the other hand AGS 290 and PI 417.213 were among the genotypes producing low yield at mid-May planting, but showed yield increase when planted early. For example AGS 290 had a yield increase of 38 % when planted early and PI 417.213 showed yield increase of 30% and 14% when planted early and late, respectively. For mid-May planting AGS 129, Ware and PI 417.310 tended to best adapted, AGS 290 and PI 417.213 for early planting, and PI 417.322 for late planting.

During the 1992 season significant differences in fructose, glucose and total sugar contents were observed among the three planting dates (Table 3). The fructose, glucose and total sugar contents in early planting was significantly higher than the mid- and late-May planting dates. In 1993 and 1994 growing seasons no significant difference was observed among the planting dates for any of three sugars analyzed. Moreover, no significant genotype x date interaction was observed for total sugar or individual sugars. This suggests that it may be possible to select genotypes for sugar content at a given planting date and to expect the same relative relationship in another planting date.

Literature Cited:

Rubel, A., Rinne, R. W. and Canvin, D. T. 1972. Protein, oil and fatty acids in developing soybean seeds. Crop Sci.12:739-741.

Tadesse Mebrahtu
Ali. Mohamed
Abdulkadir Elmi

Table 1. Mean green pod yield averaged over ten genotypes and four replications

Planting date [a]	Year		
	1992	1993	1994
Early	31002	14550	9014
Mid-May	31924	8763	7436
Late	28249	7786	7455
Mean	30392	10366	7968
CV %	19	27	23
LSD (0.05)	NS	2303	NS

[a] Early = April 30; Mid-May = May 15; Late = May 30

Table 2. Proportional green pod yield response (%) of soybeans planted early and late relative to mid-May planting

Genotype	Planting date [a]		
	Mid-May	Early	Late
AGS 129	33738	-17	-25
AGS 290	28851	+38	-03
Green & Black	27993	+09	-18
Late Giant	30644	+05	-21
Ware	34573	-30	-18
PI 416.982	31922	+02	+02
PI 417.213	23600	+30	+14
PI 417.288	30622	-11	+01
PI 417.310	45761	-21	-41
PI 417.322	31536	-08	+15
Mean	**31924**	**-03**	**-12**

[a] Early = April 30; Mid-May = May 15; Late = May 30

Table 3. Fructose, sucrose, glucose, raffinose and total sugar averaged over ten genotypes and four replications

Planting date [a]	g/ 100 g seed				
	Fructose	Sucrose	Glucose	Raffinose	Total Sugar
			1992		
Early	2.20	1.68	4.41	1.59	9.88
Mid-May	0.88	1.24	1.78	0.62	4.52
Late	0.50	0.95	0.95	0.29	2.69
Mean	1.19	1.29	2.38	0.83	5.69
LSD (0.05)	0.45	NS	1.01	NS	1.77
			1993		
Early	0.71	3.08	3.41	1.77	8.97
Mid-May	1.55	2.96	2.84	0.86	8.21
Late	1.04	3.16	2.58	0.82	7.60
Mean	1.10	3.06	2.94	1.15	8.25
LSD (0.05)	NS	NS	NS	NS	NS
			1994		
Early	0.82	1.98	2.15	0.58	5.53
Mid-May	0.73	2.76	1.70	0.98	5.17
Late	0.63	2.17	1.48	0.44	4.72
Mean	0.73	2.30	1.78	0.67	5.48
LSD (0.05)	NS	NS	NS	NS	NS

a Early = April 30; Mid-May = May 15; Late = May 30

WASHINGTON STATE UNIVERSITY
Department of Crop and Soil Sciences
Pullman, WA 99164-6420

Heritability of Yield, Plant Architecture, and Quality Traits of Edamame: the vegetable soybean

Introduction

Edamame is a vegetable-type, specialty soybean harvested immature (R_6) and eaten as a vegetable, much like green peas, or lima. It is popular in East Asia, especially Japan. Several reviews of its use, crop management, and current research have been recently published (Konovsky et al. 1994; Mebrahtu et al. 1991; Shanmugasundaram, 1991; Masuda 1989; Haas et al. 1982; Edwards & Gilbert 1981; Kline, 1980). A historical perspective is also provided by several authors (Gotoh, 1984; Smith & Van Duyne, 1951; Graff, 1949; Morse, 1941; Lloyd, 1940; Woodruff & Klaas, 1938). In the Columbia Basin of Washington State, there are records of early variety trials (Clore, 1945) and nutritional research (McGregor & Bedford, 1948).

The objective of this study was to make a preliminary evaluation of the heritability and genotype x environment interactions in the Columbia Basin for yield, plant architecture, and quality characteristics of promising edamame cultivars and land races from Japan. This information is to be used to develop a strategy for breeding and crop production research in Washington State. Successful commercial production in the Columbia Basin will only become established if long term yields and quality characteristics are stabilized by minimizing genotype x environment interactions.

Materials & Methods

Field experiments were conducted in the Columbia Basin at Washington State University's Irrigated Agriculture Research and Extension Center at Prosser (46° 15'N, 119° 50'W) in 1990 and 1991. A total of 36 edamame cultivars and land races were selected, 32 from Japan, three from the USA, and one from Taiwan. Selection was based upon earliness of maturity, uniqueness of pedigree, tolerance to cool night temperatures (Seddigh et al. 1989), seed size, availability, and popularity in Japan. Several maturity groups (MG) were represented: two genotypes are in MG 000, three in MG 00, five in MG 0, seven in MG I, twelve in MG II, four in MG III, and three in MG IV. For purposes of this study, the 36 genotypes were reclassified into three maturity groups of twelve: an early group, MG A, an intermediate group, MG B, and a late group, MG C.

On 31 May 1990 and 13 May 1991, four replications of each genotype were planted in Warden fine silt loam in four-row plots arranged as a group-balanced block design (Gomez & Gomez, 1984, page 75). Each block included the three maturity

groups; genotypes within each maturity group and maturity groups within each block were randomly assigned plot or group locations. Each four-row plot was 3 m long with a spacing of 55 cm between rows. In 1990, the trial was seeded to the desired plant density, averaging 217,241 plants/ha. In 1991, the trial was over-seeded and thinned back to the desired plant density, averaging 252,470 plants/ha. Crop management practices were in accordance with standard agronomic practices in Washington's irrigated Columbia Basin.

The different genotypes reached the R_6 stage between 15 August and 5 October in 1990, and between 2 August and 7 October in 1991. At this stage of edamame harvest, a 1.5 m length of the center two rows of each plot was cut and analyzed. The green pods were plucked from the stem using a stationary pod stripper (*Mamemogi-ki*, Kaneko Seed Co., Maebashi, Japan). Plant height, number of branches, height of the lowest pod, and the number of plants in each harvested row were measured or counted in the field. In the lab, the number and total weight of one-, two-, three-, and four-seeded pods were measured to calculate gross yield and seeds/pod; then, damaged, diseased, or defective pods were removed to calculate a net yield. Randomly selected ten-seed samples from each plot were oven dried at 30°C to calculate seed moisture and dry weight.

Pod color was measured by randomly sampling five pods from each plot and scoring their color against a white background using a calibrated chromameter (Minolta CR-200, Minolta Corp., Ramsey, New Jersey). To calculate a color value, the Hunter L*a*b system of color discrimination was employed and *X* was calculated (X = L x b / |a|) according to Chiba et al. (1989). In this system, *L* is a measure of brightness, *a* a measure of redness (+) or greenness (-), and *b* a measure of yellowness (+) or blueness (-); smaller values of *X* indicate darker green color.

The data was analyzed using the GLM and VARCOMP modules of the Statistical Analysis System with MG A, MG B, and MG C treated as separate groups. An analysis of covariance using plant density as the co-variate and a simple linear correlation analysis were run. Least significant differences (LSD) at the 5% probability level were used to compare test years and groups; Duncan's New Multiple Range Test was used to compare genotypes within groups where there were significant differences. The variance for dependent variables was calculated source by source in the ANCOVA model using the REML procedure in VARCOMP with all sources treated as random, not fixed, sources of variation (Shaw 1987). The combined variance of maturity groups and genotypes within maturity groups was compared to the total, pooled variance to estimate a broad-sense heritability.

Results

Summary statistics are presented in Table 1. An analysis of simple linear correlations between the dependent variables is presented in Table 2. A calculation of broad-sense heritability is presented in Table 3. Mean squares from the analysis of covariance are presented in Table 4.

The weather conditions during 1991 were cooler than in 1990. Mean high temperature during the growing season was 29.2°C in 1990 and 27.6°C in 1991; mean low temperature was 12.8°C in 1990 and

10.3°C in 1991. However, the first damaging frost came much earlier in 1990 and disrupted the growth of the five latest maturing genotypes. Genotypes 32-36 ranked among the lowest yielders in 1990 (5,000-10,000 kg/ha), but were among the highest in 1991 (10,000-20,000 kg/ha).

An overall strong, positive correlation existed between plant architecture characteristics. There was an inverse relationship between seeds/pod and seed weight, and a strong, positive link between gross yield and net yield. There was also a strong, positive relationship between seed weight and gross yield, much higher than any reported values to date. All dependent variables had significant genotype x environment interactions.

Heritability was higher for plant architecture, pod color, and seeds/pod than for seed weight or yield. All observed heritabilities were within published ranges except for seed weight which was much lower than reported values. Plant architecture was heavily influenced by maturity group, but yield was influenced most significantly by test year. Quality characteristics were highly influenced by test year (seed weight), maturity group (seeds/pod), and genotype (pod color).

Discussion

Seed weight is the most important quality characteristic of edamame, but in this study the broad-sense heritability was 0.10; this is low when compared to literature values that range from 0.44 to 0.94 (Table 3). The discrepancy could arise because seed weight is more malleable in the large-seeded edamame genotypes tested in this study (> 25 g/100 seeds) than in common soybean genotypes used in previous analyses (< 22 g/100 seeds).

Results might be further complicated by severe growing conditions in Washington State. Low humidity and high evapotranspiration rates in the irrigated desert of the Columbia Basin is known to cause water stress in beans (Abebe, 1977) and water stress plays a role in seed weight (Smiciklas et al. 1992). Moreover, cool night temperatures in the Pacific Northwest are also known to decrease yields by lowering seed weight under certain circumstances (Seddigh et al. 1988, 1989; Seddigh & Joliff, 1984a,b). Whatever the cause, successful commercial production of edamame in the Columbia Basin will only become established if seed weights and long term yields are stabilized through refined crop management and cultivar improvement.

John Konovsky
David W. Evans
Thomas A. Lumpkin

Literature Cited

Abebe, B. 1977. Environmental Influences on Growth of Soybeans in Central Washington. Washington State University Ph.D. Dissertation, Pullman. 150 pp.

Burton, J. W. 1987. Quantitative Genetics: results relevant to soybean breeding. *Pages* 211-47 *In* J. R. Wilcox (ed), Soybeans: improvement, production, and uses. ASA, CSSA, and SSSA, Madison.

Chiba, Y., S. Yaegashi, and H. Sato. 1989. Cultivation method of green soybean 1: effect of planting density on quality and yield. Tohoku Agricultural Research 42:279-80.

Clore, W. J. 1945. Lima beans and green edible soybeans for freezing from Central Washington. Western Canner and Packer 3:51, 53, 61.

Edwards, A. D. and L. Gilbert. 1981. Fresh Green and Sprouted Soybeans for Human Consumption. Rodale, Emmaus. 21 pp.

Gomez, K. A. and A. A. Gomez. 1984. Statistical Procedures for Agricultural Research, 2nd Edition. Wiley, New York. 680 pp.

Gotoh, K. 1984. Historical review of soybean cultivation in Japan. Tropical Agriculture Research Series 17:135-42.

Graff, H. F. 1949. The early impact of Japan upon American agriculture. Agricultural History 23:110-15.

Haas, P. W., L. C. Gilbert, and A. D. Edwards. 1982. Fresh Green Soybeans: analysis of field performance and sensory qualities. Rodale, Emmaus. 25 pp.

Kline, W. L. 1980. The Effect of Intra and Interrow Spacing on Yield of Vegetable Soybean (*Glycine max* (L.) Merr.). Cornell University Master's Thesis, Ithaca. 79 pp.

Konovsky, J., T. A. Lumpkin, and D. McClary. 1994. Edamame: the vegetable soybean. *Pages* 173-81 *In* A. D. O'Rourke (ed), Understanding the Japanese Food and Agrimarket: a multifaceted opportunity. Hayworth, Binghamton.

Lloyd, J. W. 1940. Range of adaptation of certain varieties of vegetable-type soybeans. University of Illinois Agricultural Experiment Station Bulletin 471:77-100.

Masuda, R. 1989. (Frozen vegetables--edamame). Refrigeration 64:359-76.

McGregor, M. A. and C. L. Bedford. 1948. Ascorbic acid and thiamine in fresh and frozen lima beans and soybeans. Journal of the American Dietetic Association 24:670-72.

Mebrahtu, T., A. Mohamed, and W. Mersie. 1991. Green pod yield and architectural traits of selected vegetable soybean genotypes. Journal of Production Agriculture 4:395-99.

Morse, W. J. 1941. Shanghaied...a super food. Soybean Digest 7:4-5.

Seddigh, M., G. D. Jolliff, and J. H. Orf. 1989. Night temperature effects on soybean phenology. Crop Science 29:400-06.

Seddigh, M., G. D. Jolliff, and J. H. Orf. 1988. Field evaluation of early maturing soybean genotypes for differential adaptation to low night temperatures. Crop Science 28:639-43.

Seddigh, M. and G. D. Jolliff. 1984a. Effects of night temperature on dry matter partioning and seed growth of

indeterminate field-grown soybean. Crop Science 24:704-10.

Seddigh, M. and G. D. Jolliff. 1984b. Night temperature effects on morphology, phenology, yield, and yield components of indeterminate field-grown soybean. Agronomy Journal 76:824-28.

Shanmugasundaram, S. 1991. Vegetable Soybean: research needs for production and quality improvement. AVRDC, Taiwan. 151 pp.

Shanmugasundaram, S., S. H. Cheng, M. T. Huang, and M. R. Yan. 1991. Varietal improvement of vegetable soybean in Taiwan. *Pages* 30-42 *In* S. Shanmugasundaram (ed), Vegetable Soybean: research needs for production and quality improvement. AVRDC, Taiwan.

Shaw, R. 1987. Maximum-likelihood approaches applied to quantitative genetics of natural populations. Evolution 41:812-26.

Smiciklas, K. D., R. E. Mullen, R. E. Carlson, and A. D. Knapp. 1992. Soybean seed quality response to drought stress and pod position. Agronomy Journal 84:166-70.

Smith, J. M. and F. O. Van Duyne. 1951. Other soybean products. *Pages* 1055-78 *In* K. S. Markley (ed), Soybeans and Soybean Products, Volume 2. Interscience, New York.

Woodruff, S. and H. Klaas. 1938. A study of soybean varieties with reference to their use as food. Illinois Agricultural Experiment Station Bulletin 443:421-67.

TABLE 1: SUMMARY STATISTICS

	BY YEAR			BY MATURITY GROUPING			
	1990	1991	LSD(0.05)	MG A	MG B	MG C	LSD(0.05)
Days to Flowering	50	56	—	47	53	60	—
Days to Maturity	100	110	—	89	105	121	—
Plant Height (cm)	54.6	57.8	NS	41.6	52.9	74.2	3.1
Branches/Plant	4.5	3.5	0.2	2.9	4	5.2	0.3
Lowest Pod Height (cm)	18.9	12.6	1.5	10.8	15	21.4	1.5
Pod Color (score)	140	145	3	138	147	143	3
Seeds/Pod	1.79	1.73	0.02	1.89	1.75	1.64	0.02
Seed Wt. (g/100 seeds)	20.5	29.7	1.1	21.3	28.3	25.8	0.7
Gross Yield (kg/ha)	11135	13899	927	11248	13558	12747	795
Net Yield (kg/ha)	7278	8356	585	7765	8422	7264	615

TABLE 2: SIMPLE LINEAR CORRELATION ANALYSIS

OVERALL CORRELATION COEFFICIENT

	Plant Height	Branches /Plant	Height of Low Pod	Pod Color	Seed /Pod	Seed Weight	Gross Yield	Net Yield	Gross Yield Literature (+)
Plant Height	-----	0.52 **	0.62 **	-0.06	-0.40 **	-0.03	0.17 **	-0.01	-0.28 - +0.82
Branches	-0.17	-----	0.53 **	-0.10 *	-0.32 **	-0.17 **	-0.14 *	-0.24 **	
Low Pod	0.55 **	0.02	-----	-0.17 **	-0.28 **	-0.30 **	-0.22 *	-0.28 **	
Pod Color	0.20	0.02	0.22 *	-----	-0.22 **	0.47 **	0.30 **	0.04	
Seeds/Pod	0.14	0.05	-0.08	-0.49 **	-----	-0.49 **	-0.14 *	0.29 **	
Seed Weight	-0.22 *	-0.13	0.04	0.32 *	-0.51 **	-----	0.57 **	0.21 **	-0.59 - +0.27
Gross Yield	0.21 *	-0.11	0.17	0.22 *	-0.10	0.24 *	-----	0.81 **	
Net Yield	0.22 *	-0.12	0.12	-0.03	0.22 *	0.05	0.89 **	-----	

MG A CORRELATION COEFFICIENTS

MG B CORRELATION COEFFICIENTS

	Plant Height	Branches /Plant	Height of Low Pod	Pod Color	Seed /Pod	Seed Weight	Gross Yield	Net Yield
Plant Height	-----	0.32 **	0.46 **	0.08	-0.42 **	0.13	0.22 *	-0.06
Branches	0.33 **	-----	0.30 **	-0.14	0.19	-0.39 **	-0.17	-0.11
Low Pod	0.40 **	0.42 **	-----	-0.17	0.03	-0.39 **	-0.18	-0.14
Pod Color	-0.37 **	-0.37 **	-0.39 **	-----	-0.35 **	0.46 **	0.14	-0.17
Seeds/Pod	0.14	-0.14	0.07	0.17	-----	-0.71 **	-0.39 **	0.17
Seed Weight	-0.41 **	-0.46 **	-0.73 **	0.48 **	-0.17	-----	0.57 **	0.08
Gross Yield	0.07	-0.41 **	-0.54 **	0.32 **	0.30 **	0.60 **	-----	0.68 **
Net Yield	0.14	-0.35 **	-0.39 **	0.14	0.51 **	0.36 **	0.90 **	-----

MG C CORRELATION COEFFICIENTS

SYMBOLS: MG = maturity group; ** = $p < 0.01$; * = $p < 0.05$

(+) Burton 1987

TABLE 3: BROAD-SENSE HERITABILITY

VARIANCE		Plant	Branches	Height of	Pod	Seeds	Seed	Gross	Net
	TYPE	Height	/Plant	Low Pod	Color	/Pod	Weight	Yield	Yield
Var (Y)	E	2.54	0.46	13.07	0.75	0	33.37	3321655	484855
Var (REP (Y))	E	3.79	0	0.0046	1.01	0.00003	0.24	108588	7837
Var (MG)	G	261.13	1.16	17.32	0	0.0114	0	616671	195489
Var (Y*MG)	I	0.07	0.07	19.71	16.65	0.0060	25.16	1010284	32815
Var (REP (Y*MG))	E	5.17	0	0.99	0	0	0	260262	138591
Var (G (MG))	G	140.97	0.63	9.59	107.59	0.0072	8.94	603166	0
Var (Y*G (MG))	I	33.06	0.34	13.66	67.36	0.0053	13.83	3240790	2035356
Var (Error)	E	38.45	0.80	12.58	90.92	0.0037	7.55	3195408	2161553
HERITABILITY									
OBSERVED		0.83	0.52	0.31	0.38	0.55	0.10	0.10	0.04
LITERATURE		0.66-0.90*		0.29-0.52*			0.44-0.94*	0.03-0.68*	0.22**

SYMBOLS: Var = variance; Y = year; Rep = replication; MG = maturity group; G = genotype/genetic; E = environment; I = inter

* Burton 1987

** Shanmugasundaram et al. 1991

TABLE 4: MEAN SQUARES FROM ANALYSIS OF COVARIANCE

		Plant	Branches	Height of	Pod	Seeds	Seed	Gross	Net
SOURCE	df	Height	/Plant	Low Pod	Color	/Pod	Weight	Yield	Yield
Y	1	86.40	29.84**	2344.95**	1253.82*	0.17**	3794.76**	305733941**	47807737*
Error a	6	243.32	0.35	26.30	122.38	0.0048	14.37	10329084	4110515
MG	2	26349.13**	114.55**	2821.09**	1847.71**	1.45**	1217.44**	131776508**	30891036**
MG x Y	2	222.00	6.14**	1072.17**	1185.94**	0.35**	1379.57**	65927174**	12998448
Error b	12	88.50	0.84	24.13	74.41	0.0039	4.90	6387535	3818552
G (MG A)	11	417.58**	0.75	33.61**	635.37**	0.11**	140.32**	11466298**	1076361
G (MG B)	11	516.40**	6.03**	71.19**	382.47**	0.054**	35.39**	10867531**	7278592**
G (MG C)	11	2803.62**	15.56**	327.12**	2606.06**	0.078**	309.16**	49493429**	17345685**
G (MG) x Y	33	157.00**	2.23**	69.25**	361.29**	0.025**	62.78**	16182490**	10688413**
PD	1	333.33**	2.91	116.47**	141.53	0.0042	5.49	845292	43453
Error c	197	36.95	0.79	12.05	91.80	0.0037	7.75	3207338	2172321

SYMBOLS: df = degrees of freedom; Y = year; MG = maturity group; G = genotype; PD = plant density; ** = $p < 0.01$; * = $p < 0.05$

WASHINGTON STATE UNIVERSITY
Department of Crop and Soil Sciences
Pullman, WA 99164-6420

Mapping the Pod-Shattering Trait in Soybean

Introduction

In Japan and China, soybean known as *edamame* or *maodou*, respectively, are harvested at the R_6 stage (Fehr et al., 1971) and eaten as a vegetable. In the summer, Japanese serve fresh and frozen *edamame* soybean as a snack to accompany beer, whereas in China *edamame* soybean is often found shelled in stir fry dishes. World wide production of *edamame* soybean has been inhibited by the high cost of seed ($8-15/kg). This high cost is due in part to pod-shatter, which has made it necessary to hand-harvest the seed.

Pod-shatter occurs when the dorsal and ventral sutures of the pod open and the mature seeds are dispersed (Tsuchiya, 1987). In heritability studies, the date of flowering, date of maturity, and seed size were found to have a significant correlation to pod-shatter in soybean (Caviness, 1969). Currently QTL pod-shatter research is also being conducted in Georgia (Boerma, 1996). To develop a shatter-resistant *edamame* cultivar, the genetic basis of pod-shatter has been evaluated through scoring for pod-shatter within an interspecific cross between *Glycine max* and *Glycine soja* created at Iowa State University. Significant QTL effects were found for the pod-shatter trait on linkage groups J and D in the genome.

Materials and Methods

A preliminary evaluation for mapping pod-shatter genes was conducted on 60 F2 and F3 families of an interspecific cross between A81-356022 (*G. max*) and PI 468916 (*G. soja*) grown in the summer of 1995 at an Iowa State University test plot with only one repetition. The *G. max* parent contributed the non-shattering characteristic and the *G. soja* parent contributed the shattering trait. Individual plants were scored by recording the percent pod-shatter with 0 indicating no pod-shatter and 100 signifying that all the pods had shattered. The phenotypic data was analyzed against the USDA-ARS:RFLP molecular map of the same cross (Shoemaker and Olson, 1993). 'Mapmaker-QTL' was used to analyze the data (Lincoln et al. 1992).

Results

Three QTL correlating to pod-shatter were located.

Progeny within this segregating F2:F4 population expressed a range from 0 to 100 percent pod-shatter as expected for a quantitative trait. The data indicated that three loci are primarily involved in the genetic control of pod-shatter. Two of the pod-shatter QTL mapped to the J linkage group and one locus mapped to the D

linkage group. The two QTL on linkage group J had a significant LOD score between the markers Sct65 and B_122 and the markers A_724 and A_199_2 (Fig. 1) and the locus on linkage group D between the markers B_194_2 and A_519_2 (Fig. 2):

LOD score for pod-shatter loci on the J linkage group

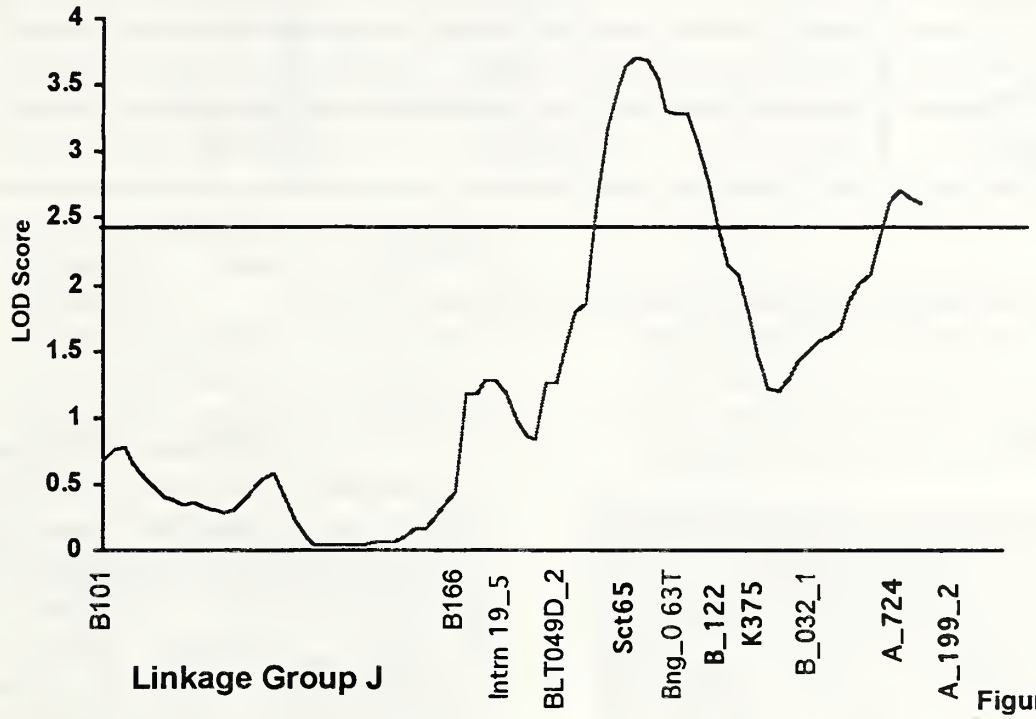


Figure 1

LOD score for pod-shatter locus on the D linkage group

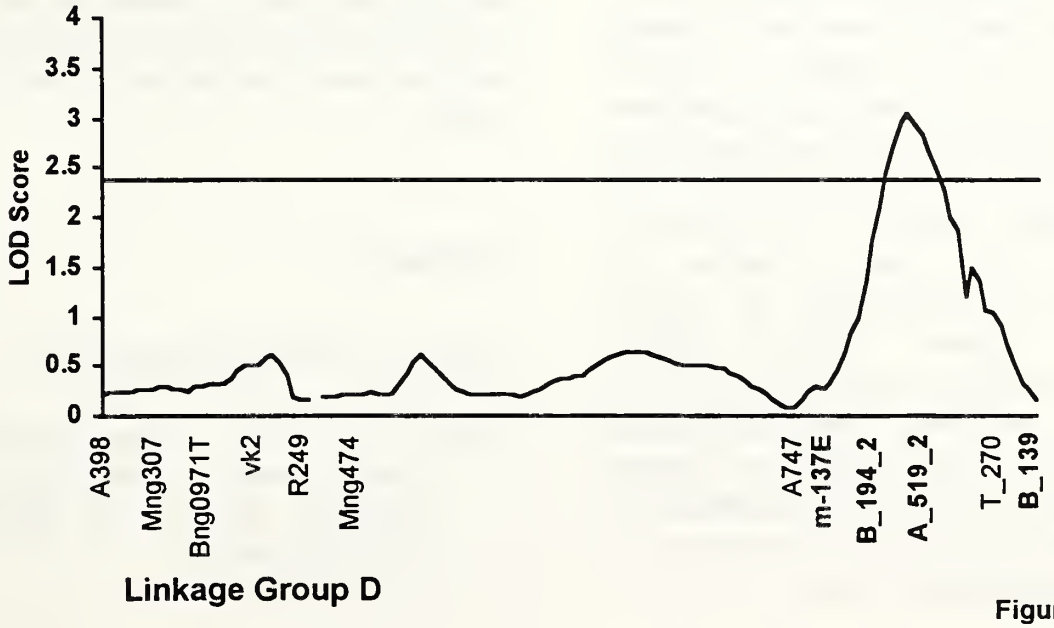


Figure 2

The non-shattering alleles were contributed by the *G.max* parent and the genetic contribution of each locus is indicated below.

	Additive effects	Dominant effects	Variance explained
Sct65 - Bng_063T	17.57%	15.21%	34.7%
A724 - A_199_2	14.10%	-4.89%	21.6%
A_519 - L161	11.95%	19.34%	23.7%

Combined the variance explained by these three QTL was 50.2%.

Discussion

To develop *edamame* soybean for large scale production, breeding programs will need to address the problem of pod-shatter. The QTL data collected from the interspecific cross of *G.max* and *G.soja* indicates that there are three main genes controlling pod-shatter located on the J and D linkage groups. In spite of the preliminary nature of this data, since each QTL detected had a significant correlation with the shattering trait, locating three main genes was possible. However, due to the small population evaluated, some QTL may have been missed or the effect of the QTL may have been overestimated. With the availability of molecular markers, this knowledge will facilitate the development of shatter-resistant *edamame* soybean cultivars. However, epistasis or linkage-drag effects of QTL for shattering and more details regarding the QTL influencing edamame vegetable quality need to be determined so that vegetable quality is not removed while selecting non-shattering lines.

In this preliminary evaluation, the QTL explained a little over half the observed variance, but environmental factors such as relative humidity and changeable wet and dry weather also play a significant role in pod-shatter (Caviness, 1963). The understanding of the genetic components controlling pod-shatter will help to develop a non-shattering *edamame* soybean cultivar, making mechanical harvest of edamame seed possible, and thus the subsequent decrease in the price of seed will make mechanized edamame seed production economically feasible.

References

Boerma, R., Personal Communication.

Caviness, C. E. 1963. A Physiological and Genetic Study of Shattering in the Soybean. University of Missouri, Ph.D. Dissertation.

Caviness, C. E. 1969. Heritability of pod dehiscence and its association with some agronomic characters in soybeans. Crop Science 9: 207-209.

Fehr, W. R., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stages of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Science 14: 929-931.

Lincoln, S. E., M. Daly, E. S. Lander. 1992. Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1 Tech Rep. 2nd ed. Whitehead Institute for Biomedical Research, Cambridge, MA.

Shoemaker, R. C. and T. C. Olson. 1993. Molecular linkage map of soybean p. 131-139. In S. J. O'Brien (ed.) Genetic maps: Locus maps of complex genomes. 6th (ed.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Tsuchiya, Takehiko. 1987. Physiological and genetic analysis of pod shattering in soybeans. Japan Agricultural Research Quarterly 21(3): 166-175.

L. A. Saxe[1]
C. Clark[2]
S. F. Lin[2]
T. A. Lumpkin[1]

[1] Washington State University, Crop and Soil Sciences, Pullman, WA 99164-6420
[2] Iowa State University, Department of Agronomy, Ames, IA 50011

I would like to acknowledge the IMPACT Center at Washington State University for its financial support, Feng Han for his efforts, and Randy Shoemaker for consultation and access to the USDA map and mapping population.

Index of Authors